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Development of a Sugar Beet Processing Laboratory

A. I. Morgan, E. J. Barta, and G. O. Kohler[1]

Received for publication November 6, 1958

The Western Regional Research Laboratory, Albany, California, has developed a processing line capable of continuously producing sucrose from sugar beets. Funds supplied by the Beet Sugar Development Foundation, Fort Collins, Colorado, under a cooperative agreement with the U. S. Department of Agriculture have paid for a large share of this development.

The purposes of this project were three: to provide a means of relating beet composition to processing quality, to evaluate new steps or variations in traditional sugar beet technology, and to provide intermediate and final products of known history for laboratory bench studies. Of these three purposes, the first was dominant. Scale and type of apparatus were mainly determined by this consideration.

Long-term average changes in sugar beet composition are caused by changing soil fertilization practice, by differences in stage of maturity at harvest, by development of new varieties, or by new harvesting methods. The effects of these changes can be evaluated only in equipment similar to factory systems, in which conditions are under exact control.

The process is shown in Figure 1. The purposes of the various steps are the same as those for equivalent steps in sugar beet factories.

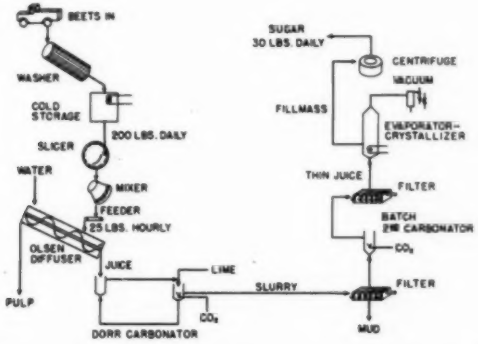

Figure 1.—Sugar beet processing laboratory at the Western Regional Research Laboratory, Albany, Calif.

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[1] Western Regional Research Laboratory, Albany, California, a laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

The capacity of the processing laboratory equipment must be such as to composite a beet sample of significant size. This size depends on which chemical component of the beet is being considered. The coefficients of variation differ considerably. The minimum sample size agreed upon is 100 beets or about 200 lbs. For 95% certainty, 100 beets selected at random will be representative of a large population within 10% in regard to sucrose, marc, total anions, oxalic acid, total nitrogen, malic acid, raffinose, amino nitrogen, and galactinol. The coefficient of variation will exceed 10% only slightly in the case of glutamate (4, 11)[2]. Figure 2 shows coefficients of variation observed in random sampling of a large population for the principal components of sugar beets versus sample size. The population includes beets of two varieties grown in three different states under two levels of fertilization. This study will be separately reported by Dr. R. L. Hurst and others (11).

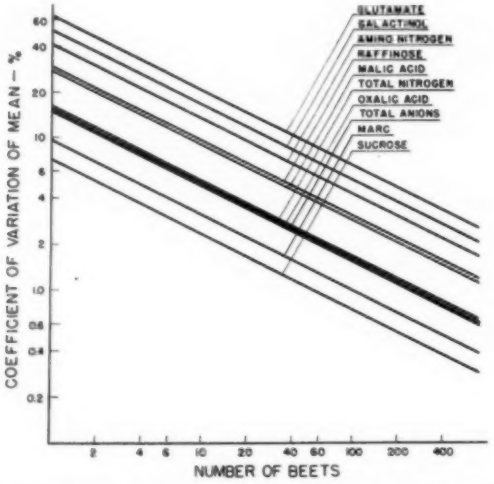

Figure 2.—Relation of sample size to coefficient of variaton of means for random sampling.

Although 100 beets as a daily composite is a minimum, the processing laboratory design should not demand a much larger quantity since the convenient size for experimental planting and sampling techniques is close to 100 beets for each condition. Because of start-up and shut-down losses, the rate of processing of beets should be the minimum for the 100-beet sample, compatible with an eight-hour work day. In accordance with these

[2] Numbers in parentheses refer to literature cited.

conditions, the laboratory was designed for 20 pounds of beets per hour. Such a scale, 1/5000 of factory, is too small for evaluating processing equipment directly and demonstrates the subordination of the secondary objectives to composition studies.

Continuous operation of the diffusion, carbonation, and evaporation units was considered essential. The remaining steps —washing, slicing, filtration, second carbonation, and crystallization—were designed for batch operation. The continuous steps were matched and integrated. The apparatus was sized so that retention times were suitable at the chosen flow rate. Accurate control of all conditions for each step was emphasized in design. Facilities for measurement and analysis were as extensive as the processing equipment itself.

Truck load lots of field or experimental beets are washed by tumbling them through high-pressure sprays inside a horizontal rotating cylinder composed of widely spaced steel slats. About 2 tons of beets per hour can be cleaned. The beets are packed in moist wood shavings in 40-pound lots in wooden boxes (2). If the beets delivered are intended for several experiments, the washed beets are sorted into size classes, and a proportionate weight of each class is packed in each box. This compensates for composition trends with beet size. A fungicide dip improves storage life.

The boxes of beets are stored in a forced-air-circulation cold room which is maintained at 1° C. Boxes are removed for experiment according to a random sequence. Beets are washed free of shavings and then sliced.

The cossette cutter, shown in Figure 3, is a three-foot drum rotating at 155 r.p.m. in the vertical plane. Around the drum are spaced 14 standard 46 division cossette knives, one knife to a block. The tangential knife speed is about factory average, 20 ft./sec. Beets are forced against the knives by a volute curving to a small angle with the knife drum. The length of a perfect cossette, containing 100 grams of beet, can be calculated for any knife setting. Real cossettes have a greater length per 100 grams, since they have less than the maximum possible cross-section dimensions. The actual length observed was found to be constant at 170% of the calculated length. Typical cossettes are comparable to factory cossettes when sharp knives are used, i.e., 22 meters per 100 grams for 2.25 mm. up and back knife settings.

About 50 kg. lots of cossettes are then tumbled in Tygon-lined, covered, concrete mixer in the cold room. After 10 minutes, successive 50 gm. samples have the same variation in sucrose

content by polarization as portions of one 50 gm. sample subsampled after blending.

The cossettes are fed to a small Bruniche--Olsen continuous countercurrent diffuser, which, as modified, has proved very satisfactory on the job (3). This unit consists of a round-bottomed copper trough, 6 inches wide, 4 ft. long, containing a single interrupted scroll without breaker bars (see Figure 4). A given

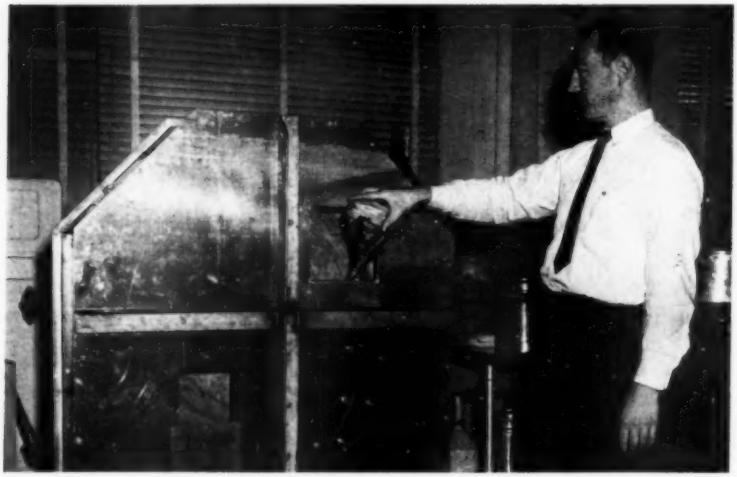

Figure 3.—Cossette cutter.

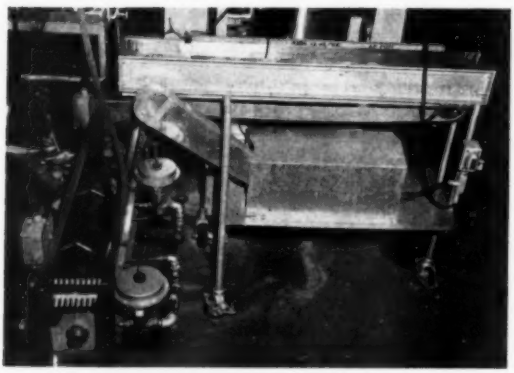

Figure 4.—Extractor and feeder.

weight of cossettes is spread between marks on a constant-speed belt which drops them through an opening in the cover at the low end of the diffuser. Exhausted pulp is lifted over a weir at the high end by means of a rotating notched plate. Preheated distilled water is metered into the trough bottom at the high end, and juice leaves through a perforated, wiped plate at the low end. Temperature is maintained by jackets, the steam to which is controlled by two bulbs in the cossette mass. The cold cossettes are raised to the diffusion temperature of 70° C. within three minutes or four inches of travel.

Various important factors affecting the operation of this Olsen diffuser were evaluated in a series of experiments. Each factor was fixed at the level resulting in the largest number of theoretical contacts (1). An equivalent dimensionless figure, Silin's constant (8), is better known in the beet sugar industry. Optimum conditions according to this basis are the following: A trough slope of 1° to horizontal, liquid level at scroll shaft height, scroll speed of 1⅓ r.p.m., and beet retention time of 40 minutes. The attack angles of the interrupted flights of the scroll were adjusted to produce the same retention time with beet feed rates which varied from 9 to 12 kg./hr. Sucrose remaining in the exhausted pulp ranged from 0.10 to 0.30% for percentages of juice rate to beet rate from 140 to 120. Table 1 shows the low relative efficiency of the Olsen laboratory diffuser compared to other units. These figures are all based on our own observations.

Table 1.—Relative Efficiencies of Diffusers.

	No. Theoretical Contacts	Silin's Constant
Laboratory Olsen, Albany, Calif.	10	4.0×10^{-5}
Laboratory Roberts, Albany, Calif.	13	5.2
Factory Roberts, Alvarado, Calif.	16	6.7
Factory Olsen, Eaton, Colo.	20	9.1

The labor saving, the true countercurrent action, and especially the reproducibility dictated the use of the Olsen diffuser for experimental work despite its inefficiency. In a long series of experiments with different feeds, the efficiency of this unit had a standard deviation of 0.4 theoretical contact.

The hot diffusion juice flows into a modified model Dorr carbonator which is based on a development of R. A. McGinnis at Spreckels Sugar Company (9). A schematic drawing of the carbonator is shown in Figure 5. This device continuously limes

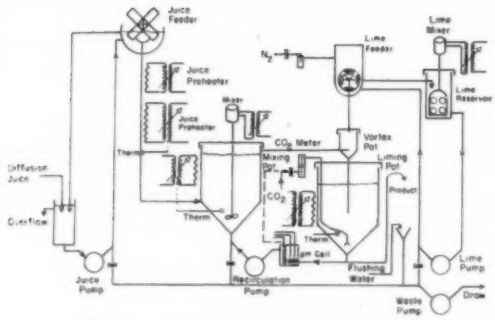

Figure 5.—Model Dorr carbonator.

and carbonates up to 3 gallons of diffusion juice per hour. Automatic temperature and pH control are provided. Homogenized milk of lime and bottled carbon dioxide are used. The details of this apparatus together with examples of measurements and analyses are given in another report (6).

First carbonation slurry is batch filtered in Buchner funnels, through paper. The filtrate is saturated with carbon dioxide for 5 minutes while boiling, then boiled for 3 minutes without further gassing. The second carbonation juice is again filtered through paper to yield "thin" juice suitable for evaporation (5).

Thin juice can be concentrated continuously in a 3-cubic-foot evaporator-crystallizer, shown in Figure 6. This is a vertical stainless-steel cylinder 14 inches in diameter, 4 ft. 6 in. high, connected by 4-in. tubing to a direct contact condenser and a two-stage vacuum system. The evaporator is drained through a 3-in. plug cock. Proof stick samples may be removed for analysis.

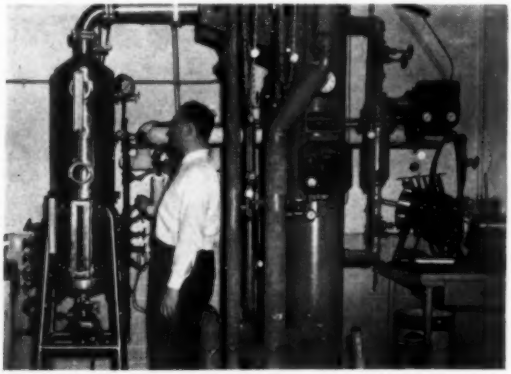

Figure 6.—Evaporator-crystallizer and vacuum system.

Steam may be used in the jacketed bottom and in any of 5 spiral submerged coils, totaling 6 square feet of heating surface. Volumes from 0.3 to 30 gallons can be boiled under pressures from 1.3 to 30 inches Hg absolute at evaporation rates up to 450 lbs. water per hour. Large head space and an entrainment separator permit handling of foaming liquids. The performance of this evaporator has been good and may be predicted by

$$U = 22 \left(\frac{\Delta t N}{\mu} \right)^{0.3} \left(\frac{1}{1 - .027h} \right)$$

where U is overall heat transfer in Btu/hr./ft.^{2}/°F.

(Δt) is overall temperature difference in °F.

N is number of surfaces in use.

μ is boiling liquid viscosity in centipoises.

h is average liquid height above hot surface in inches.

Crystals can be produced in the evaporator, or the nearly saturated juice can be canned and stored. Such juice can later be evaluated as to crystallization velocity and ultimate sucrose solubility in laboratory-scale dilatometers (10).

Four men are now required to operate the laboratory, one of whom must be highly skilled in all operations. This excludes labor for some analytical services such as moisture determination or Kjeldahl and Van Slyke nitrogen measurements, but does include labor for alkalinities, lime analysis, percent refractive dry substance, polarizations, sedimentation rates, filtration rates, colors, lime salts, colloid content, and pH.

The laboratory is in operation at present (7). Considerable additional equipment and parts, however, are necessary, and will be acquired in order to improve reliability and to reduce the excessive labor needed. New equipment is being built to embody proposed improvements on traditional processing steps. Such proposals will be evaluated by comparison with the standard line described.

Summary

A very small pilot plant for crystal sugar production from sugar beets has been construed in order to measure the effects of changing beet composition on processing qualities. Continuous extraction, purification, and evaporation were adopted. The rate of continuous processing is limited by the diffusion and carbonation steps. This limiting rate was chosen by consideration of the minimum beet sample size which represents a very large number of beets with respect to the most variable known component within the beet. In order to process this sample con-

veniently in one day, the throughput chosen was nine kg. of beets per hour. The number of ideal contacts within the model diffuser is compared with commercial diffusers. Operation of the model carbonator and evaporator is described.

Acknowledgments

The authors wish to acknowledge the ideas and efforts during this long project of many people in the Engineering and Development Laboratory and in the Field Crops Laboratory of the Western Utilization Research and Development Division.

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Fractionation of Nitrogen Compounds in Beet Molasses

J. B. Stark, A. E. Goodban, and R. M. McCready[1]

Received for publication December 26, 1958

For many years the sugar beet industry has been interested in the "harmful nitrogen" content of processing juices. "Harmful nitrogen" is defined here as those nitrogen-containing compounds that are not removed during processing and appear eventually in molasses. These compounds are believed by many sugar beet technologists to be especially deleterious in beet sugar manufacturing operations and to cause the production of more than normal amounts of molasses. Whether or not this is the case, more information on sugar-beet nitrogen compounds seems desirable, since they account for nearly 50 percent of the total weight of non-sugars in molasses.

As part of our molasses composition studies, taken during the 1952 campaign, samples were obtained from 9 factories: 6 operating straight houses—Betteravia, Clarksburg, and Manteca, California; Brighton, Colorado; Carrollton, Michigan; and Moorhead, Minnesota—and 3 operating Steffen houses—Alvarado and Woodland, California; and Toppenish, Washington. Nitrogen determinations were made on the various fractions separated by ion exchange resins. Information is presented relating to some of the known nitrogen compounds as well as the unidentified nitrogen compounds.

The nitrogen compounds of molasses were separated into 3 fractions referred to as basic, acidic, and unadsorbed, depending on their reaction with ion exchange resins under the conditions of this experiment. Diluted molasses was passed in turn through a strongly acidic cation exchange resin in the (H^+) form. Basic nitrogen compounds are defined as those that were adsorbed from a dilute molasses solution by the (H^+) cation exchange resin. Since this fraction contains many amphoteric compounds, most of the amino acids in addition to more basic compounds such as betaine are present.

The effluent from the cation exchanger mentioned above was then passed through the column of (intermediate strength) anion exchange resin in the chloride-formate form. Only the strongly acidic compounds were retained. Nitrate and pyrrollidone carboxylic acid (PCA) are the principal nitrogenous components of the acidic fraction that are retained on the resin.

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[1] Western Regional Research Laboratory, a laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture. Presented at meeting of American Society of Sugar Beet Technologists, Detroit, Mich., Feb. 4-6, 1958.

There are traces of at least two unidentified nitrogen-containing acids present.

Unadsorbed nitrogen compounds are defined as those compounds that were not adsorbed from the solution by either resin. Under the conditions of the experiment this fraction contains weakly acidic nitrogen compounds and may contain weakly basic compounds that were not retained by the cation resin and some neutral nitrogen compounds. This fraction also contains sucrose, other soluble carbohydrates, and soluble neutral compounds. If pectin or pectic acid were present in the original molasses they would appear in this fraction. Although these substances are acidic their high molecular weights prevent their adsorption by ion exchange resins.

Experimental Methods

The columns used for separation of the basic fraction were glass tubes 60 cm. long and 4 cm. in diameter. One end of each was stoppered with a one-hole rubber stopper containing an outlet tube which could be closed with a clamp or stopcock. A circle of fine-mesh nylon bolting cloth was used to cover the stopper and outlet tube to prevent loss of resin. The columns were loaded with 400 ml. of commercial-size Dowex-50 (H)[2] (Dow Chemical Company, Midland, Michigan) in a water slurry. As determined by previous trials, this is 5- to 10-fold the amount necessary to remove the basic components.

The columns used for separation of the acidic fraction were glass tubes 60 cm. long and 1.6 cm. in diameter with the type of closure described above. The lower part of the tubes were filled with about 50 ml. of an intermediate-strength anion exchange resin Permutit A (Permutit Corporation, New York, N.Y.) equilibrated with hydrochloric acid to pH 2.5. Fifty ml. of the resin equilibrated with formic acid to pH 2.8 was placed above this. These columns were to fractionate the organic acids of molasses including lactic, glycollic, PCA, citric, and others (1) and to provide a fractionation of nitrogen compounds into the two classes, acidic and unadsorbed.

Figure 1 shows a diagram of the separation procedure. Fifty grams of molasses, diluted to 200 ml. with water, was passed through the column of Dowex-50 (H) cation exchanger at the rate of one liter per hour. The column was washed with water until the pH of the effluent was 3.3. The volume of the acidic effluent (I) including the wash water was about 1300 to 1500 ml. Three liters of 10 percent ammonium hydroxide was passed

[2] Mention of specific products does not constitute endorsement by the Department of Agriculture over others of a similar nature not mentioned.

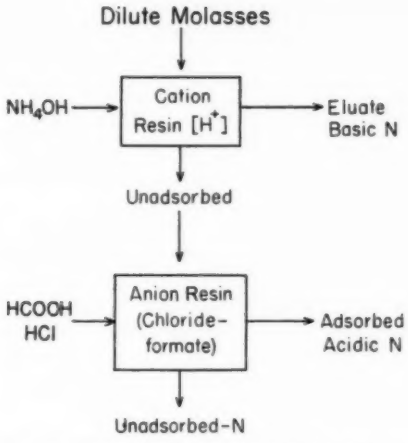

Figure 1.—Separation of nitrogen fractions with ion exchange resins.

through the column of cation resin to elute the basic nitrogen compounds (II). The ammonia was removed by boiling the solution vigorously while the pH was maintained above 7 by additions of sodium hydroxide. The alkaline condition of the ammonium hydroxide eluate of the cation resin degraded most of the amides present to form additional ammonia. This ammonia and the ammonia originally present in the molasses were lost during concentration of this fraction.

An aliquot of the acidic effluent from the cation exchanger (I) containing about 20 milliequivalents of anions, was passed through a column of anion resin (Permutit A) in the chloride-formate form. The column was then washed with 500 ml. of water. The combined effluent and wash form the unadsorbed fraction (III). The anion column was fractionally eluted with a mixture of dilute formic acid hydrochloric acids to obtain the fraction containing PCA (IV) (1).

The methods of analysis used were previously described (2). The original molasses was analyzed for total, amino, amide, and ammonia nitrogen and for betaine. The basic fraction (II) was analyzed for total, amino, amide, and ammonia nitrogen. The acidic effluent (I) from the cation column containing the acidic and neutral substances was analyzed for total and amino nitrogen. Later experiments have shown that the Kjeldahl method used for total nitrogen recovered only 60 to 80 percent of the nitrogen present. As a consequence the total nitrogen values for molasses may be low by four percent and the acidic

fraction by eight percent in samples with a high nitrate content. The unadsorbed fraction (III) was analyzed for total nitrogen. The fraction IV eluted from the anion column with dilute formic-hydrochloric acid contains mostly PCA. It was analyzed for total nitrogen to give estimate of the PCA present.

Results

The classification of the nitrogen compounds into basic, acidic, and unadsorbed is arbitrary and depends upon the operating conditions used in the experiment. For example, the anion exchanger in the hydroxyl form would absorb some of the weakly acidic compounds that passed through the resin column when the resin was in the chloride-formate form. Under the conditions of low pH caused by release of mineral acids in the cation exchanger some weakly basic compounds or amphoteric compounds pass through and are found in the acidic or unadsorbed fractions. Most of the nitrogen compounds found in the unadsorbed fraction could have been recovered on an ion exchange resin by recycling the solution through a column of cation resin in the hydrogen form and then passing the effluent through a column of anion resin in the hydroxide form. The known compounds in the basic fraction are mainly betaine and amino acids.

Since only part of the nitrogen compounds were eluted from the anion resin, total nitrogen present in the acidic fraction was calculated by difference between the nitrogen in acidic effluent (I) and the nitrogen in neutral effluent (III). PCA and nitrate comprise most of the acidic nitrogen although there are traces of at least two unidentified nitrogen compounds that were eluted by hydrochloric acid and there are probably others that are eluted only with difficulty if at all.

The unadsorbed fraction is composed mainly of weakly acidic nitrogen compounds such as uracil and uridine that are not retained by the anion resin. It may also contain some weakly basic compounds that passed through the cation resin as a result of the low pH of the effluent and some nearly neutral nitrogen compounds.

Table 1 shows the amount of molasses nitrogen in the basic, acidic, and unadsorbed fractions. The average distribution shows 66 percent of the nitrogen in molasses as basic, 28% as acidic, and 6 percent as unadsorbed.

Table 2 shows the distribution of basic nitrogen among the known compounds and classes of compounds and also the quantity that has not yet been identified. The basic nitrogen fraction averages 49 percent betaine, 27 percent amino acids.

Table 1.—Basic, Acidic, and Unadsorbed Molasses Nitrogen (g. nitrogen/100 g. non-sucrose solids).

Factory[1]	Basic	Acidic	Unadsorbed
Be	4.55	1.98	0.33
Br	4.33	1.76	.43
CaM	4.42	1.53	.39
Cl	4.52	1.79	.39
Ma	5.05	2.34	.58
Mo	3.94	1.83	.34
Al[2]	4.15	1.99	.42
To[2]	4.91	2.25	.44
Wo[2]	3.24	1.27	.43
Average	4.35	1.86	.42

[1] Abbreviations for the factories are as follows: Al—Alvarado, Be—Betteravia, Br—Brighton, CaM—Carrollton, Cl—Clarksburg, Ma—Manteca, Mo—Moorhead, To—Toppenish, and Wo—Woodland.

[2] Steffen molasses.

Table 2.—Distribution of Basic Nitrogen in Molasses (g nitrogen/100 g. non-sucrose solids).

Factory[1]	Betaine	Van Slyke Amino	Amide	Ammonia	Remainder[3]
Be	2.02	1.34	0.28	0.12	0.66
Br	1.77	1.03	.15	.05	1.20
CaM	2.34	1.04	.17	.04	.70
Cl	2.28	1.06	.20	.09	.74
Ma	2.29	1.40	.25	.10	.88
Mo	2.27	1.00	.20	.05	.29
Al[2]	1.56	1.16	.19	.06	1.05
To[2]	2.43	1.29	.25	.08	.73
Wo[2]	1.65	.81	.20	.07	.38
Average	2.07	1.13	.21	.07	.74

[1] See Table 1 for abbreviations.

[2] Steffen molasses.

[3] Does not include an estimated 0.13 g. purine-pyrimidine nitrogen.

7 percent amide and ammonia, and 17 percent unidentified. Included in the unidentified portion is nitrogen from purines and pyrimidines which are known to be present but were not determined in these samples. On the basis of the analysis of one sample of Manteca molasses (3) for individual purines, pyrimidines, and nucleosides, the N due to these compounds would account for .13 g. of the unidentified .74 g. of N.

The acidic nitrogen distribution is given in Table 3. From the averages, PCA accounts for 55 percent and amino compounds 6 percent of the acidic nitrogen. A large part of the remainder

Table 3.—The Distribution of Acidic Nitrogen in Molasses (g nitrogen/100 g. non-sucrose solids).

Factory[1]	PCA	Van Slyke Amino	Remainder
Be	1.17	0.10	0.71
Br	.90	.13	.73
CaM	75	.11	.69
Cl	1.02	.09	.68
Ma	1.46	.13	.75
Mo	.98	.14	.71
Al[2]	.67	.08	1.24
To[2]	1.58	.10	.57
Wo[2]	.69	.11	.47
Average	1.02	.11	.73

[1] See Table 1 for abbreviations.

[2] Steffen molasses.

is due to nitrate, which averaged 0.55 g. of nitrate nitrogen per 100 g. of impurities for 17 molasses samples from the 1956 campaign. If we accept this as an approximation for the 1952 molasses, however, we must make a correction, since the total N analysis would have measured only 70 percent of this, or 0.38 g. Using this figure, there is still 19 percent of the acidic nitrogen which is unknown. The acidic amino nitrogen is also unknown, but probably includes glutamic and aspartic acids which leaked through the cation resin because of the high acidity, pH 1.5, of the effluent.

Table 4.—Total and Unidentified Molasses Nitrogen (g. nitrogen/100 g. non-sucrose solids).

		Unidentified				
Factory[1]	Total	Basic	Acidic	Unadsorbed	Total	% of Original
Be	7.19	0.66	0.68	0.33	1.67	23
Br	6.09	1.20	.70	.43	2.33	38
CaM	6.40	.70	.66	.39	1.75	27
Cl	6.79	.74	.65	.39	1.78	26
Ma	8.47	.88	.72	.58	2.18	26
Mo	6.44	.29	.68	.34	1.31	21
Al[2]	6.37	1.05	1.21	.42	2.68	42
To[2]	7.61	.73	.54	.44	1.71	22
Wo[2]	5.59	.38	.44	.43	1.25	22
Average	6.75	.74	.70	.42	1.86	28

[1] See Table 1 for abbreviations.

[2] Steffen molasses.

Table 4 gives the relationship between total and unidentified nitrogen in the basic, acidic, and unadsorbed fractions. An average of 28 percent of the total nitrogen appears to be unidentified. If a correction for the nitrate in the acidic fraction is made, based on the average nitrate content of the 1956 campaign molasses samples, 22 percent of the nitrogen remains unidentified. If these unknown compounds average 20 percent nitrogen, about 7.5 percent of the non-sugars in molasses are unidentified nitrogen compounds. If the average nitrogen content of the compounds is as low as 10 percent, then 15 percent of the total non-sugars are made up of this group of unidentified compounds. The true value probably lies between the extremes of 7.5 and 15 percent.

Acknowledgment

The authors wish to express their appreciation for the generous cooperation of the beet sugar companies in supplying the samples for this work. They are indebted to Marian Long for determinations of total, ammonia, and amino nitrogen, to Earl Potter for determination of amide nitrogen, and to Roberta Erlandsen for determination of betaine.

Summary

A method using ion exchange resins is described that enables the separation of molasses nitrogen compounds into basic, acidic, and unadsorbed classes of nitrogen compounds.

Nitrogen compounds make up about 50% of the nonsucrose molasses solids and their average distribution is 66% basic, 28% acidic, and 6% unadsorbed. The 6% unadsorbed is mainly weakly acidic compounds. Weakly basic, or neutral compounds may also be present.

Forty-nine percent of the basic nitrogen occurs in betaine, 27% in amino acids, 7% as amides and ammonia, and 17% remains unidentified. Acidic nitrogen is distributed as 55% PCA, 6% amino compounds, 20% nitrate, and 19% unidentified. The compounds containing unadsorbed nitrogen have not been identified.

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Genetic Improvement of Processing Quality in Sugar Beets[1]

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In the past few years the improvement of processing quality in sugar beets has become of paramount importance to the beet sugar industry. The purpose of this article is to present information on the possibility of genetic improvement of processing quality in sugar beets.

Sugar Content

No critical data are available to adequately explain the inheritance of sucrose content. It has been generally assumed (for lack of evidence to the contrary) that sucrose percentage is conditioned by additive factors or genes, with no expression of dominance or heterosis for sucrose content. This assumption has been substantiated (over the years) by the results obtained from rigid selection for high sucrose content. These selections have resulted in so-called "sugar type" varieties that are high in percent sucrose but low in yield of roots and sugar per acre. The intermediate sucrose content of the first male-sterile hybrids, or top crosses, further supported the belief that sucrose content was conditioned by simple additive genes and that dominance or heterosis for sucrose content did not exist.

Current studies, however, conducted with inbred lines and their F_1 hybrids have provided evidence which strongly suggests the existence of dominance and even heterosis for sucrose content in sugar beets. These findings are of major importance to all breeding programs designed to utilize hybrids or synthetic varieties, and they forecast significant advances to be made in raising the sucrose percentage of commercial beets.

Table 1 lists the results of a preliminary test conducted in 1956 by Powers (unpublished data) with inbred lines and their F_1 hybrids. These data show that the sucrose content of the F_1 hybrids is not intermediate. With one exception the sucrose content of all F_1 hybrids exceeded the average of the respective parents and in most cases were not statistically different from the sucrose content of the higher parent. Specifically, hybrids

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1, 2, and 3 show complete phenotypic dominance while hybrids 5, 6, 7, 8, and 9 show partial dominance for higher sucrose content. Hybrid 4, on the other hand, indicates complete phenotypic dominance for low sucrose content.

Table 1.—Percentage Sucrose for F_1 Hybrids and Inbred Parents, 1956, U. S. Department of Agriculture.

Hybrid No.	Pedigree	P_1	P_2	F_1	$\frac{P_1 + P_2}{2}$
1	50-406 X 52-430	18.5	18.0	18.8	18.2
2	50-406 X 52-307	18.5	17.2	18.7	17.8
3	52-430 X 52-307	18.0	17.2	18.1	17.6
4	52-408 X 53-1278	18.3	17.4	17.4	17.8
5	50-406 X 52-414	18.5	14.2	17.9	16.4
6	52-430 X 52-414	18.0	14.2	17.8	16.1
7	52-414 X 52-305	14.2	18.5	17.4	16.4
8	52-414 X 52-307	14.2	17.2	16.4	15.7
9	51-319 X US 201	9.2	17.4	15.1	13.3

At the 0.05 level the value of t is 1.63 percent.

Evidence for the existence of heterosis with regard to sucrose content is shown in Table 2. These data, from current work by Powers et al. (6)[3] on population genetic studies with sugar beets at different levels of soil fertility, show that the F_1 hybrid has one percent more sugar on the fertilized plots than the highest parent (52-307). The odds against this difference being due to chance are well in excess of 99:1. This, therefore, demonstrates heterosis for increased sucrose content on the fertilized plots.

Table 2.—Population Means for Percentage Sucrose, Fertilized and Non-Fertilized Plots, 1956, U. S. Department of Agriculture.

Population	Fertilized	Non-Fertilized
	%	%
50-406 (P_1)	16.1	17.4
F_1 hybrid	17.6	17.6
52-307 (P_2)	16.6	16.5

Recent studies by Rush and Oldemeyer (7) on combining ability in sugar beets which involved 90 F_1 hybrids and their respective parents likewise showed clear-cut evidence of dominance and heterosis for sucrose content.

In these studies the F_1 hybrids, as a class, were not intermediate with respect to sucrose content. Slightly more than

[3] Numbers in parentheses refer to literature cited.

one-third of the F_1 population showed either phenotypic dominance or heterosis for increased sucrose percentage. The average sucrose percentage of the F_1 hybrids was 14.47%, while that of the female parents was 14.08% and that of the pollen parents was 13.86%. Moreover, the mean sucrose content of the F_1 hybrids was significantly higher at odds of 99:1 than the mean sucrose percentage of either the female or pollen parents.

Table 3.—Percentage Sucrose for Selected F_1 Hybrids and Inbred Parents, Amalgamated Sugar Company.

Hybrid No.	Pedigree	P_1	P_2	F_1	$\frac{P_1 + P_2}{2}$
1	A2-9OHO X 5-188	13.92	13.84	14.80	13.88
2	175 H15 X 5-205	14.90	13.92	15.55	14.41
3	E21 X 5-239	14.12	13.32	14.82	13.72
4	E322 X 5-207	14.22	14.19	15.00	14.20
5	A2-9OHO X 5-231	13.92	13.90	14.75	13.91
6	E21 X 5-231	14.12	13.90	14.65	14.01
7	175 H15 X 5-188	14.90	13.84	15.26	14.37
8	A2-9OHO X 5-204	13.92	12.54	14.36	13.23
9	113H3 X 5-238	13.25	13.91	14.31	13.58
10	175 H15 X 5-237	14.90	13.88	15.09	14.39

LSD at 5% level = 0.62%. 1% level = 0.84%.

Table 4.—Percentage Sucrose for F_1 Hybrids and Their Inbred Parents, Holly Sugar Corporation.

Hybrid No.	Hybrid	P_1	P_2	F_1	$\frac{P_1 + P_2}{2}$
1	5152-01 X 6112-0	14.56	14.23	15.28	14.40
2	CT9MS X 6112-0	14.38	14.23	14.92	14.31
3	5152-01 X 6113-0	14.56	13.75	14.70	14.16
4	CT9MS X 6114-0	14.38	13.56	14.48	13.97

LSD at 5% level = 0.52%

Typical cases of heterosis and dominance for increased sucrose percentage are shown in Tables 3 and 4. In Table 3, hybrids 1 to 5, inclusive, illustrate heterosis; the sucrose content of the F_1 hybrids is significantly higher than the sucrose content of the highest parent. Hybrids 6 to 10, inclusive, demonstrate phenotypic dominance; the sucrose content of the F_1 hybrids is significantly greater than the average sucrose content of their respective parents. In Table 4, hybrids 1 and 2 show heterosis for percentage sucrose and hybrids 3 and 4 show complete dominance.

The findings regarding heterosis and dominance for sucrose content are of great importance to the beet sugar industry. They

not only have a decided bearing on breeding programs, particularly those designed to utilize hybrids and synthetic varieties in the commercial production of sugar beets, but they also assure, within the near future, greater production of sugar per acre from beets significantly higher in sucrose content and improved processing quality.

Sodium

Data showing the possibilities for changing sodium content of the beet root by selection are presented in Table 5. An examination of the figures in the last column reveals that the high-sucrose, low-sodium selections resulted in a decrease in the sodium content of the beets. This was true for both the American

Table 5.—Selection for Sodium Content of the Beet Root.

Sugar Company and Selection	Sugar per Acre	Beets per Acre	Sucrose	Na. Sodium
	Lbs.	Tons	%	%
American Crystal				
Check	8251	26.9	15.32	0.0572
Hi-su, Hi-na	7868	24.7	16.01	0.0554
Hi-su, Lo-na	8137	25.3	16.05	0.0314
LSD, 5%	638	2.1	0.46	0.0084
Great Western				
Check	6468	20.4	17.7	0.042
Hi-su, Hi-na	6537	20.1	18.1	0.043
Hi-su, Lo-na	6420	19.4	18.4	0.023
LSD, 5%	263	0.7	0.3	0.005

Crystal and Great Western selections. Likewise in both cases the selections for high percentage sucrose resulted in an increase in this character, whether the accompanying selection was for high or low sodium. Selection for high sodium and high sucrose did not result in an increase in sodium above that of the check. However, data not listed in Table 5 showed that selection for high sodium produced strains having this characteristic. There was no increase in production of sugar per acre because the increase in percentage sucrose was offset by a corresponding decrease in tons of beets per acre.

The conclusions drawn concerning sodium are as follows:

1. Selection pressure readily affects the sodium content of beets in either direction.
2. In most beet populations, sodium and sucrose content are negatively correlated to a rather high degree.

3. Actually the sodium characteristic of the sugar beet is of questionable importance to the breeding program, unless a low-sodium variety is extremely important from the processing standpoint.
4. The value of low sodium content per se in beets for processing also is questionable, since the quantity of soda ash added for control of lime salts far exceeds the quantity found in the beet.

This was true for both sets of data, American Crystal and Great Western. For further data and discussion see (2), (3), (4), and (5).

Thin-Juice Purity

The summarized data for thin-juice purity are presented in Table 6. In all cases listed in Table 6, selection for greater purity has resulted in an increase in this character. This was true whether the work was done by the Holly Sugar Corporation or the Great Western Sugar Company. In the case of the Great Western selections this increase in purity was accompanied by an increase in percentage sucrose, and a corresponding decrease in yield of beets per acre. However, in the case of selection 732 made from GW 359, the decrease in yield of beets per acre did not offset all the increase in purity and percentage sucrose, and there was a net gain in tons of sugar per acre.

Table 6.—Selection for Thin-Juice Purity.

Sugar Company and Selection	Sugar Per Acre	Beets	Sucrose	Purity
	Lbs.	Tons	%	%
Holly				
Check	5495	18.4	15.0	92.87
Hi purity	5461	18.5	14.8	93.50
Lo purity	5686	19.5	14.6	92.00
LSD, 5%				0.68
Great Western				
Check, 359	5726	21.4	15.8	92.31
Hi purity, 732	5994	20.8	16.7	93.29
LSD, 5%	136	0.4	0.2	0.22
Check, 359	5898	21.5	16.1	92.64
Hi purity, 738	5884	20.5	16.6	93.40
LSD, 5%	216	0.7	0.2	0.37

Raffinose

The results of selection for percentage raffinose on dry substance in the press juice and accumulation of raffinose in storage are shown in Figure 1.

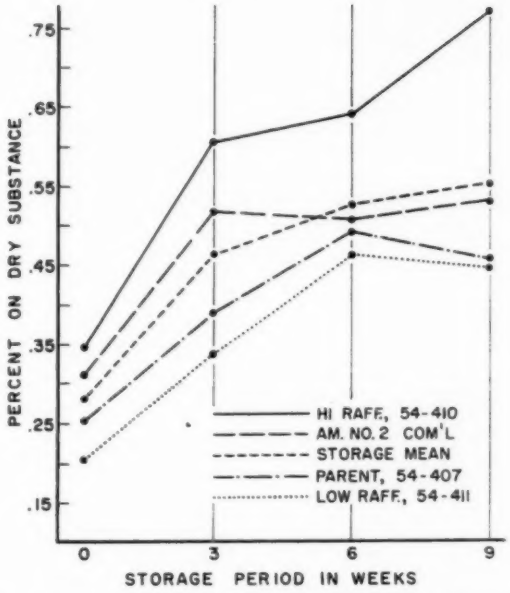

Figure 1.—Selection for percentage raffinose on dry substance in the press juice and accumulation of raffinose in storage, American Crystal Sugar Company.

Strain 54-407 was the result of a random selection of mother beets from the same parent strain (American No. 1) as selections 54-410 and 54-411. The latter two were selected for high and low raffinose content, respectively.

No significant differences for raffinose content were found between beets stored in a regular storage pile and those stored in the root cellar. However, all varieties showed a significant increase in raffinose content during storage, as shown in Figure 1. The percent increase for the low-raffinose selection compared with the high-raffinose selection was approximately equal after nine weeks of storage.

Wood (8) reports that progeny tests of varieties B619 and B620 selected from GW 359 demonstrated the heritability of raffinose percentage. The low-raffinose selection B619 did not accumulate raffinose, in storage tests, at as great a rate as did either the parent or the high-raffinose selection B620. Some inbred lines in the cooperative indexing program have been found to have zero content of raffinose. Studies on the inherit-

ance of raffinose production showed that the high parent and low parent were differentiated by five gene pairs.

The relation between percent sucrose, percent raffinose on dry substance in the press juice, tons of beets, and percentage galactinol on dry substance in the press juice at different dates of harvest is shown in Figure 2.

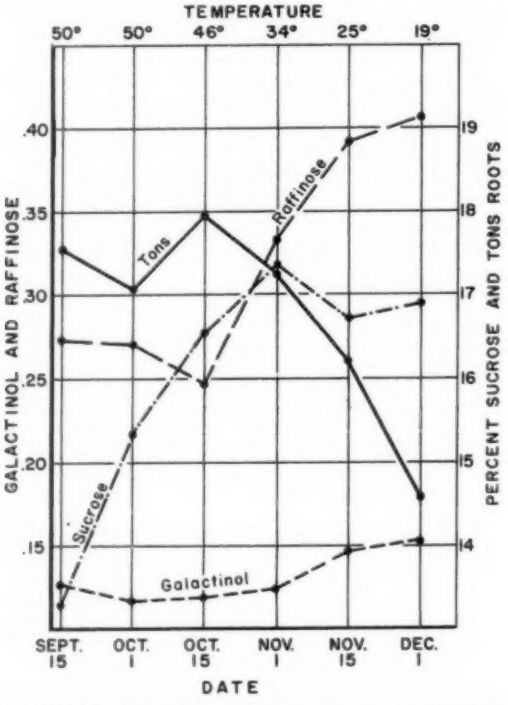

Figure 2.—Relation between percentage sucrose, percentage raffinose on dry substance in the press juice, tons of beets per acre, and percentage galactinol on dry substance in the press juice at different dates of harvest, American Crystal Sugar Company.

The decrease in tons of beets for the later harvest was due to freezing of the beets. It can be seen that both percentage sucrose and percentage raffinose increased between October 1 and November 1. Finkner from this finding postulates that since both sucrose and raffinose increased during the test period, each must be produced by its own physiologic system, and that raffinose is not necessarily a polymerized product of sucrose degradation.

Finkner's postulation on the independence of production of sucrose and raffinose in the beet may be examined further as follows:

First, both the data of Finkner presented in this manuscript (Figure 1) and evidence presented previously by Wood (8) and Brown and Wood (1) indicate that raffinose content continues to increase after the beets are harvested and stored. Such an increase in raffinose content must be at the expense of one or more of the other materials in the root and may result from (a) polymerization of smaller molecules or (b) degradation of larger molecules.

In either case there should be measurable intermediate products.

Second, raffinose and sucrose both rotate the plane of polarized light in the same direction; i.e., to the right. Raffinose, however, is 1.85 times as effective in this respect as is sucrose; therefore, even if sucrose is the basic substance destroyed to form raffinose, the loss would probably not be detected by means of the polariscope. Desiccation encountered during storage also tends to increase polarization. In our studies we have not shown differences in polarization during ordinary storage periods beyond what might be expected as result of errors in measurement and respired amounts. It is known, of course, that sucrose is used up by respiratory activity in stored beets.

Raffinose accumulation in 6 field-grown varieties at 6 dates of harvest is shown in Figure 3.

A study of Figure 3 reveals that from October 15 to November 15 all strains and varieties increased in percentage raffinose on dry substance in the press juice. From November 15 to December 1 there was no further increase in raffinose content for some strains, whereas for others increase in this chemical continued.

Association of Chemical Characteristics in Sugar Beets

The correlation coefficient data pertaining to the association of chemical characteristics in sugar beets are provided by the Amalgamated Sugar Company.

The correlation coefficients showing the association between total nitrogen in the beets and in the thin juice, press juice and diffusion juice, and between these and monosodium glutamate extract are listed in Table 7.

In every case the association is high, indicating that a positive or negative change in one is accompanied by a proportionate and corresponding change in all the others. Total nitrogen in the beet is highly correlated with nitrogen in the different juices:

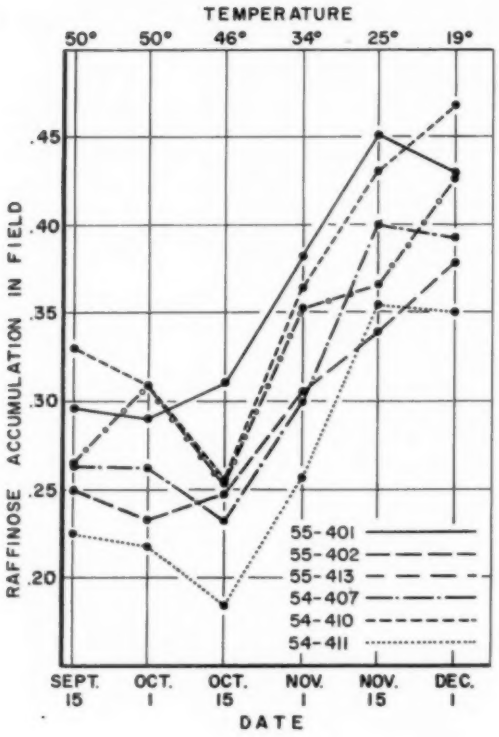

Figure 3.—Percentage raffinose on dry substance in the press juice accumulation in six field-grown varieties at six dates of harvest, American Crystal Sugar Company.

Table 7.—Simple Correlation Coefficients Based on 168 Pairs of Observations Within Seven Varieties and Three Nitrogen Levels for Total Nitrogen in Beets and Different Juices and Monosodium Glutamate, Amalgamated Sugar Company.

Variate	Amino N in			Monosodium Glutamate
	Thin Juice	Press Juice	Diff. Juice	
N in beets (lbs/ton)	0.93	0.92	0.94	0.89
Amino N, thin	.....	0.96	.....	0.91
Amino N, press	.....	.....	.....	0.89
Amino N, diff.	.....	0.96	.....	0.91

thin, press, and diffusion. Also, N in the press juice is very highly correlated with N in the thin juice and N in the diffusion juice.

This raises the question whether one total N determination would suffice, which would almost have to be true with regard to association with other characters. This is borne out by the correlation coefficients with MSGE. There are no significant differences between the correlation coefficients between any of the nitrogen determinations and MSGE. The correlation coefficients between the four nitrogen determinations listed in Table 7 and percentage sucrose were —0.56, —0.56, —0.52, and —0.57, respectively. This finding was equally valid for seven other chemical characters with which these four nitrogen determinations were correlated. Hence in the discussion which follows, for the sake of simplification and clarity, only total N in the beet will be considered in relation to other characters.

The correlation coefficients between total nitrogen in the beet and 12 other characters are shown in Table 8.

Table 8.—Simple Correlation Coefficients Based on 168 Pairs of Observations Within Seven Varieties and Three Nitrogen Levels for Total Nitrogen and 12 Other Characters, Amalgamated Sugar Company.

Variate	N in Beets (lbs/ton)	Variate	N in Beets (lbs/ton)
Sucrose, harvest	—0.56	Potassium, root	0.52
Sucrose, cossettes	—0.58	Sodium, root	0.70
Purity	—0.87	Ash, juice	0.64
Extraction	—0.73	Non sugars	0.88
Raffinose	—0.19	Yield	0.56
P_2O_5, beets	0.04	NO_3, petiole	0.64

The correlation coefficients between total nitrogen in the beets and percentage sucrose at time of harvest and percentage sucrose in the cossettes are high (—0.56 and —0.58) as compared with those found between characters in most biological material. The association is negative. Percentage purity and percentage extraction are even more highly correlated with total nitrogen in the beets. Again the association is negative. Since these four characters (percentage sucrose at harvest, percentage sucrose in the cossettes, percentage purity, and percentage extraction) are not independent of each other, that all should show similar relations with total nitrogen in the beets is to be expected.

The correlation coefficients between total N in the beets and raffinose and P_2O_5 are not of sufficient magnitude to indicate that any appreciable association exists.

Total nitrogen is positively correlated with potassium, sodium, ash, non-sugars, yield, and nitrate nitrogen in the petioles. The relation is positive. The degree of association for sodium and non-sugars is very high, while that for other characters is high.

The correlation coefficients for percentage sucrose and 10 other characters are listed in Table 9.

Table 9.—Simple Correlation Coefficients Based on 168 Pairs of Observations Within Seven Varieties and Three Nitrogen Levels for Percentage Sucrose at Harvest and 10 Other Characters, Amalgamated Sugar Company.

Variate	Sucrose at Harvest	Variate	Sucrose at Harvest
Yield of roots	—0.36	Ash, juice	—0.46
N in beets	—0.56	Non-sugars	—0.57
NO_3, petiole	—0.64	P_2O_5, (lbs/ton)	0.24
Sodium, root	—0.65	Purity	0.68
Potassium, root	—0.47	Extraction	0.75

The association between percentage sucrose and yield of roots, total N in the beets, nitrate nitrogen in the petioles, sodium, potassium, ash, and non-sugars is negative. The degree of association may be considered as average for yield and high for the other characters showing a negative relation with percentage sucrose. P_2O_5, purity, and extraction are positively associated with percentage sucrose, and the degree of association is rather high for purity and extraction.

The correlation coefficients for yield and nine other characters are listed in Table 10.

The only significant negative association is between yield and percentage sucrose, and the degree of intensity is not great. P_2O_5 and raffinose show very little if any relation with yield. Nitrogen, sodium, potassium, amino nitrogen, ash, and MSGE are positively associated with yield. With the exception of sodium, the degree of association is high.

Table 10.—Simple Correlation Coefficients Based on 168 Pairs of Observations Within Seven Varieties and Three Nitrogen Levels for Yield of Beets per Acre and Nine Other Characters, Amalgamated Sugar Company.

Variate	Yield	Variate	Yield
Sucrose, harvest	—0.36	Amino N, juice	0.54
P_2O_5 (lbs/ton)	—0.05	Ash, juice	0.50
N in beets	0.56	MSGE	0.53
Sodium, root	0.32	Raffinose	0.12
Potassium, root	0.48		

In evaluating the above correlation coefficients in relation to the bearing they may have on the possibility of altering chemical characters and recombining them by breeding procedures, the method of calculating the correlation coefficients must be kept in mind. They were calculated on the basis of 168 pairs of plot values within seven varieties and three nitrogen levels. Hence they are an average association of affects attributable to replications, varieties, and fertilizer treatments.

The means of percentage sucrose, parts per million NO_3-N in the petioles, and weight per root for the interaction of replications X populations X treatments are listed in Table 11. The following interpretation of these data is taken from Powers, Robertson, Whitney, and Schmehl (6).

For all five replication groups the percentage sucrose of the F_1 exceeds that of A54-1 on the fertilized plots, while the reverse is true (except for the 33-40 group) on the non-fertilized plots. For the first three groups 50-406BB, the F_1, and 52-307 show an increase in percentage sucrose on the fertilized plots as compared with the non-fertilized plots; whereas the reverse is true for populations A54-1, A54-1BB, and 50-406.

The parts per million of NO_3-N in the petioles involving the above comparisons are of interest. On the non-fertilized plots A54-1 has an average of 588 parts per million of NO_3-N in the petioles for the first 3 groups and the average percent sucrose is 18.2. The same values of the F_1 for the first 3 groups on the fertilized plots are 630 and 18.3. Apparently the F_1 and A54-1 have the same optimum concentration of nitrogen in the petioles for maximum percentage sucrose in the roots. However, the F_1 reaches this optimum at the high fertility level, whereas, A54-1 reaches approximately this same concentration of NO_3-N at the lower fertility level. It seems that either A54-1 is more efficient in taking up nitrogen at the lower fertility and retains it in the petioles, or at the higher fertility the F_1 uses more nitrogen in metabolism, or does not retain it in the petioles. This difference in reaction of populations may have a decided bearing on breeding varieties and hybrids better adapted to sugar production at higher fertility levels.

Another comparison involving 50-406BB with A54-1BB and A54-1 for the first three groups and the fertilized and non-fertilized treatments is of interest. An average increase in NO_3-N in the petioles from 486 parts per million on the non-fertilized plots of 50-406BB to 1296 on the fertilized plots was not accompanied by a decrease in percentage sucrose, 17.8 to 17.9, whereas for A54-1BB and A54-1 the corresponding values are 634 to 1308

Table 11.—Means of Percentage Sucrose, Parts per Million NO_3-N in the Petioles, and Weight per Root for the Interaction of Replications X Populations X Treatments (from Powers, Robertson, Whitney, and Schmehl, 6).

Population and Character	Fertilized Replication Groups					Non-Fertilized Replication Groups				
	1-8	9-16	17-24	25-32	33-40	1-8	9-16	17-24	25-32	33-40
A54-1										
Sucrose, percent	17.1	17.6	17.7	16.8	14.6	18.0	18.4	18.3	18.2	16.6
NO_3-N, ppm	1724	1350	829	3484	13778	671	452	640	864	6055
Weight, lbs.	2.90	2.55	2.41	2.44	2.69	1.93	1.66	1.61	2.02	2.43
A54-1BB										
Sucrose, percent	16.9	18.2	17.9	16.2	14.5	17.8	18.7	18.3	17.5	17.0
NO_3-N, ppm	1505	1154	1264	5368	15132	499	702	702	826	5231
Weight, lbs.	2.85	2.57	2.51	2.66	2.61	1.82	1.51	1.47	1.62	2.13
50-406BB										
Sucrose, percent	17.6	18.1	18.1	17.0	15.5	17.4	18.0	17.9	17.7	16.8
NO_3-N, ppm	1482	1050	1357	2656	14791	500	406	554	569	5426
Weight, lbs.	2.09	2.24	2.07	1.82	1.96	1.44	1.43	1.20	1.35	1.58
50-406										
Sucrose, percent	16.8	16.8	16.6	15.6	14.7	17.9	17.8	17.7	17.3	16.4
NO_3-N, ppm	702	551	632	788	8812	530	436	359	538	2948
Weight, lbs.	1.11	1.09	0.98	0.96	0.98	0.79	0.61	0.68	0.79	0.87
F_1 hybrid										
Sucrose, percent	18.2	18.5	18.2	17.0	16.0	17.6	18.3	17.7	17.6	16.8
NO_3-N, ppm	852	390	648	2082	11328	234	374	524	398	4890
Weight, lbs.	2.29	2.49	2.19	2.03	2.16	1.60	1.39	1.20	1.67	1.82
52-307										
Sucrose, percent	16.6	17.4	17.6	16.4	14.9	15.7	17.3	16.6	17.4	15.6
NO_3-N, ppm	570	578	866	1817	14392	546	390	374	826	4126
Weight, lbs.	1.21	1.18	1.10	1.10	1.19	0.58	0.45	0.41	0.67	0.81

accompanied by sucrose values of 18.3 to 17.7 and 588 to 1301 accompanied by sucrose values of 18.2 to 17.5. These results indicate that certain populations may be able to tolerate more NO_3-N in the petioles than others without an accompanying reduction in percentage sucrose. Finally, the second order interaction of replications X population X treatments is of interest in connection with information it furnishes concerning the possible nature of heterosis in this hybrid. Both the F_1 and the inbred parent 52-307 on the fertilized plots produced higher percentage sucrose for the first three replication groups than they did for these same replication groups on the non-fertilized plots. The reverse is true for 50-406, the other inbred parent, as regards these same replication groups. The percentage sucrose for both the F_1 and 52-307 is less on the fertilized plots than on the non-fertilized plots for the last two replication groups. Thus the behavior pattern for the F_1 and 52-307 is the same, as regards the ability to produce higher percentage sucrose at the higher level of soil fertility. Hence, this ability to produce high percentage sucrose at the higher fertility level is partially or completely dominant. On the other hand the 50-406 inbred potentially is the higher percentage sucrose parent. This is shown by a comparison of 50-406 and 52-307 on the non-fertilized plots. The ability of 50-406 to produce a high percentage sucrose is at least partially if not completely dominant in the F_1 hybrid. The ability of the F_1 to react as the inbred parent 50-406 in respect to high potential percentage sucrose production and as the 52-307 parent at the higher fertility level results in the heterosis of the F_1 grown on the high-fertility plots; that is, the F_1 exceeds either parent in percentage sucrose on the fertilized plots.

Further study of the percentage sucrose values of Table 11 reveals that the behavior pattern of 50-406BB as compared with 50-406 on the fertilized and non-fertilized plots is very similar to the behavior pattern of the F_1 as compared with 50-406. The populations 50-406 and 50-406BB differ from each other in that the seed producing the plants of 50-406BB came from mother beet plants of 50-406 which at the time of flowering were exposed to pollen from 22 other varieties, strains, and inbreds. This evidence is important because it lends support to the finding that heterosis for percentage sucrose exists, and indicates that it is not of too rare occurrence in sugar beets.

Another comparison of interest involves the relation between these three characters in replication group 1-8 and replication group 33-40. As can be determined from a study of Table 11

there is a decrease in both percentage sucrose and weight per root of group 33-40 compared with group 1-8 on the fertilized plots. Also there is a decided increase in parts per million of nitrate nitrogen in the petioles of group 33-40 as compared with group 1-8. Under conditions of this experiment the application of excesses of nitrogen results in a decided reduction in percentage sucrose and a slight reduction in weight per root.

Summary and Conclusions

1. Heterosis for percentage sucrose in F_1 hybrids produced from certain inbreds was found independently by three investigators, each working under the diverse climatic conditions found in three different States—Colorado, Idaho, and Wyoming. Also, dominance or partial dominance of higher percentage sucrose was found to be of rather frequent occurrence. These findings regarding heterosis and dominance for sucrose content are of considerable importance to the beet sugar industry. They provide the information needed for producing hybrids and synthetic varieties of higher sucrose content. Hence, these findings assure for the industry within the near future greater production of sugar per acre from beets significantly higher in sucrose content and improved processing quality.

2. The data on the inheritance of sodium, potassium, and raffinose demonstrate that these chemical constituents are subject to change by breeding procedures. That is, they can be either increased or decreased in inbreds, hybrids, and varieties according to the dictates of thee beet sugar industry.

3. Studies on the relations between yield of roots per acre, percentage sucrose, and chemical constituents show that these characters, both chemical or agronomic, can be recombined into desirable combinations. Recombination within the nitrogen group of characters may be the exception, as the positive relation between the different nitrogenous compounds was rather marked.

The fact that covariance accounted for only a relatively small amount of the total variance of characters other than the nitrogenous compounds and the fact that these characters were not closely correlated with the nitrogenous compounds indicate that desirable recombination of chemical constituents can be attained.

4. The population genetic studies at different levels of soil fertility show that populations differ in their ability to produce higher percentage sucrose at the higher nitrogen fertility level. Hence it seems that the plant breeder can aid in the solution of

the problems encountered because of the application of greater amounts of nitrogenous fertilizers. It should be possible for the plant breeder to produce varieties and hybrids of sugar beets that will yield higher percentage sucrose at the higher levels of nitrogen concentration in the soil than do the varieties now being grown. However, it should be pointed out that such improved varieties and hybrids probably will not be able to completely overcome the harmful effects of the indiscriminate use of nitrogenous fertilizers. These improved hybrids and varieties must be accompanied by reasonable fertilizer practices if their high quality potentials are to be realized.

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Terminal Oxidases of the Mature Sugar Beet Root[1]

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Few reports concerning the identity or relative activity of the terminal oxidase enzymes of the sugar beet root have been published. Phenol oxidase has been shown to be active in the leaves of the sugar beet by Arnon (1)[3], and Dawson and Tarpley (4) also detected laccase activity. Bhagvat and Hill (2) included the sugar beet root in an extensive list of plants tested spectrophotometrically for cytochromes and cytochrome oxidase. The bands of cytochromes a, b, and c were observed but no cytochrome oxidase activity was detected.

Stout and Spikes (10) described the effects of various chemical inhibitors on the oxygen consumption of sugar beet root tissue discs. Lack of inhibition by 8-hydroxyquinoline, coupled with inhibition by cyanide and azide led the authors to the presumption that respiration in the sugar beet root is mediated by way of a cytochrome oxidase or polyphenolase rather than by ascorbic acid oxidase. No direct evidence of cytochrome oxidase participation was present, however.

The experiments described below were designed to investigate the active terminal oxidases of the sugar beet root through Qo_2 effects of particular inhibitors and substrates added to a standard respiratory medium, the use of carbon monoxide inhibition, and spectrophotometric techniques for the detection of cytochrome oxidase activity.

Materials and Methods

Sugar beet seed, SKE-R-11 (supplied by the British Columbia Sugar Refining Company, Vancouver, B. C.) was sown in flats in the greenhouse on February 23, 1957 and uniform seedlings planted in the field May 11, 1957. Beginning July 15, beets whose diameters were five inches or over, were taken at random for the oxidase studies.

After the roots had been cleaned and trimmed, a trans-section 3.75 cm. in height was cut from each root just below the region of greatest diameter. Trans-slices one mm. in thickness were cut from these cylinders by a Spencer hand microtome and from

[1] Investigation supported by extramural research grant EMR-50 of Science Service, Canada Department of Agriculture.

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[3] Numbers in parentheses refer to literature cited.

these slices discs one cm. in diameter were cut by a steel cork borer, avoiding the periphery and the core of the beet. The discs (one cm. in diameter, one mm. thick) were thoroughly mixed in distilled water and after 30 minutes removed and rinsed with 50 ml. of the medium described below.

Twenty discs (average fresh weight ca 1.62 g., dry weight ca 0.29 g.) were blotted dry, quickly weighed, and placed in a Warburg vessel containing a total of 4.0 ml. of solution whose composition was 0.4 M sucrose, 0.05 M phosphate buffer at pH 6.8, and 0.04 M KCl. Inhibitors, selected from those suggested by James (8), when used, were included in the medium. Substrates, when used, were placed in the sidearm and added at zero time. Carbon dioxide was absorbed by 0.2 ml. of 20 percent KOH (or of 2.0 M. KCN in the cyanide inhibition trials) on fluted filter paper in the center well.

After 15 minutes equilibration, the oxygen consumption at 25° C. was determined at 20 minute intervals for periods up to two hours. The rate of shaking was 120 strokes per minute. Respiration rates were computed as microliters of oxygen consumed per hour per gram fresh and per gram dry weight of the discs. Because of their parallelism, the rates are reported on the fresh weight basis only.

In the determinations of carbon monoxide inhibition the gas atmosphere of the reaction flasks was changed using the evacuation method of Burris (3). An atmosphere of 95% CO and 5% O_2 was used and the rate of respiration in the carbon monoxide was compared with that obtained in a mixture of 95% N_2 and 5% O_2. Since CO is absorbed in small amounts by KOH, a control containing no tissue was used.

The desirability of the spectroscopic method of determining cytochrome oxidase activity has been re-emphasized by Fritz and Beevers (5). Cytochrome c was dissolved in 0.03 phosphate buffer (pH 7.3) at the rate of 0.5 mg./ml., and reduction was accomplished by adding sodium hyposulfite. The excess hyposulfite was oxidized by intermittent shaking of the solution for 20 minutes. Sugar beet root tissue was homogenized in a Potter-type homogenizer with an equal weight/volume of cold 0.03 M phosphate buffer (pH 7.3). The homogenate was centrifuged at 1000 g for five minutes and the supernatant used as the enzyme source. To 2.5 ml. of the reduced cytochrome c solution 0.5 or 0.05 ml. of the enzyme preparation was added. Measurement of absorbance at 550 mμ was made at room temperature with a Beckman Model B spectrophotometer.

The effect of pH upon ascorbic acid oxidation was followed in an attempt to determine whether an increase in oxygen consumption obtained by the addition of ascorbic acid as substrate to sugar beet root tissue discs were the result of increased activity of laccase or of ascorbic acid oxidase. Root tissue was homogenized in an equal weight/volume of cold 0.4 M sucrose and centrifuged at 1000 g for five minutes. The supernatant was used as a source of enzyme. To 2.9 mls. of a 5.0 mM solution of ascorbic acid in 0.03 M phosphate buffer over a range of pH values from 4 to 8, 0.1 ml. of the enzyme preparation was added. The changes in optical density were followed at 265 mμ with a Beckman Model DU spectrophotometer at room temperature.

Results

Manometric determinations were replicated up to four times. At least four respirometers were used for each treatment at each replication. The average Qo_2 and percentage inhibition or activation in each replication is given in the tables, but analysis of variance involved all the individual readings of the respirometers.

Inhibitors

The results obtained with the inhibitors at a concentration of 0.01 M are given in Table 1.

Cyanide and azide: In the respiratory medium containing cyanide, the oxygen uptake by the sugar beet tissue slices was reduced 92.2 percent. The inhibitions, as determined by four replications involving different beets, were 90.4, 89.9, 91.1, and 87.4 percent. The average inhibition obtained with sodium azide was 85.0 percent. Cyanide and azide strongly affect metal-containing enzymes, particularly the iron-containing cytochromes and the copper-containing oxidases. Ascorbic acid oxidase, however, appears insensitive to azide. The high sensitivity to cyanide and azide suggests that metallo-oxidases are the ones primarily active in the respiration of sugar beet root.

Diethyldithiocarbamate (Dieca): Dieca is a powerful inhibitor of the copper enzymes. The average inhibition produced by the presence of dieca in the respiratory substrate was 37.7 percent. This is positive evidence that copper enzymes are active.

Resorcinol: Resorcinol, which is a competitive inhibitor of phenolase, (polyphenoloxidase) produced a reduction of 22 percent in oxygen consumption, compared with the respiration of discs in the control medium. The value is significant at the 0.01 level.

Thiourea: In two replications, involving five respirometers each, the presence of thiourea resulted in a lowering of the

Table 1.—Effect of Inhibitors on the Respiration of Sugar Beet Root Tissue Discs. Respiration (Microliters 0_2/Hour/Gram Fresh Weight)

Inhibitor	Replicates 1 Control	1 With Inhibitor	1 % Inhibition	2 Control	2 With Inhibitor	2 % Inhibition	3 Control	3 With Inhibitor	3 % Inhibition	4 Control	4 With Inhibitor	4 % Inhibition	Average % Inhibition	Level of Significance
Cyanide	77.4	7.4	90.4	85.0	8.6	89.9	84.3	7.5	91.1	69.5	8.8	87.4	92.2	.001
Azide	67.7	11.2	83.4	72.8	10.7	85.5	77.9	13.7	86.2				85.0	.001
Dieca	54.5	32.8	40.0	62.3	39.0	37.4	60.4	38.6	36.1				37.8	.001
Resorcinol	61.4	46.6	24.1	60.6	48.6	20.0							22.0	.01
Thiourea	68.1	65.1	4.4	60.6	56.4	7.0							5.7	N.S.[1]
8-hydroxyquinoline	66.0	65.8	0.2	64.0	63.0	1.6							0.9	N.S.
CO (light)	67.0	57.5	14.0	36.4	28.4	22.0	81.1	71.9	11.0	66.9	57.8	13.0	15.0	.01
CO (dark)	67.0	60.9	9.1	36.4	27.0	26.0	81.1	68.1	16.0	66.9	57.6	14.0	13.8	.01
CO (light vs. dark)														N.S.

[1] Almost significant at the .05 level.

respiratory rate. The two average inhibition values were 4.4 and 7.0 percent, giving an over-all average of 5.7 percent. This is almost, but not quite, significant at the 0.05 level. Thiourea is an inhibitor of such copper enzymes as ascorbic acid oxidase, phenolase, and laccase.

8-hydroxyquinoline: In the two replications 8-hydroxyquinoline did not reduce the rate of oxygen consumption. The average increase of 0.9 percent lacked significance. This inhibitor is active against ascorbic acid oxidase. The result obtained, which is similar to that reported by Stout and Spikes (10), casts doubt on the presence or activity of ascorbic acid oxidase.

Carbon monoxide: Respiration rates in an atmosphere of 95% N_2 and 5% O_2 did not differ significantly from the rates in air. Compared with rates in the 95% N_2/5% O_2 atmosphere the mixture 95% CO/5% O_2 produced an average decrease in respiratory rate of 15 percent in light, and 13.8 percent in the dark. Both values are significant at the 0.01 level. No significance exists between the inhibition in light and in darkness. The results tend to question the participation of cytochrome oxidase in the respiratory sequence.

Taken together, the results obtained with the inhibitors suggest 1.) metallo-oxidases are involved in sugar beet root respiration, 2.) ascorbic acid oxidase is non-active or absent, 3.) there is no evidence of cytochrome oxidase activity and 4.) the active oxidases are probably copper-containing enzymes.

Substrates

The substrates were added to the respiratory medium at the rate of 0.5 or 1.0 mg./ml. The effects on oxygen uptake by sugar beet root discs are given in Table 2.

The laccase substrates, hydroquinone and p-phenylenediamine, increased the rate of oxygen utilization by an average of 11.5 and 30.5 percent, respectively. The values are significant at the 0.05 and 0.01 levels.

The addition of protocatechuic acid and tyrosine, substrates for phenolase, did not result in as great increments in oxygen consumption. Although the average increase occasioned by protocatechuic acid was 11.5 percent, this value just lacked significance at the 0.05 level. The average increase, 9.0 percent, produced by the addition of tyrosine was significant at the 0.05 level. Catechol, which serves as a substrate for both laccase and phenolase, produced an average increase of 32.7 percent in respiration. This was significant at the 0.01 level.

Table 2.—Effect of Substrates on the Respiration of Sugar Beet Root Tissue Discs. Respiration (Microliters 0_2/Hour/Gram Fresh Weight)

		Replicates							
		1			2				
Substrate	Mg ml	Control	With Substrate	% Activation	Control	With Substrate	% Activation	% Average Activation	Level of Significance
Hydroquinone	0.5	65.5	70.0	7.0	63.8	73.9	16.0	11.5	.05
p-phenylenediamine	0.5	65.5	87.8	34.0	63.8	81.2	27.0	30.5	.01
Protocatechuic acid	1.0	87.6	99.6	14.0	70.5	76.8	9.0	11.5	N.S.[1]
Tyrosine	0.5	67.0	72.9	9.0				9.0	.05
Catechol	1.0	87.6	95.1	8.0	70.5	110.8	57.0	32.7	.01
Glycolic acid	1.0	73.5	71.1	—3.2	58.1	49.1	—15.1	—9.2	N.S.
Lactic acid	1.0	74.5	70.7	—5.1	82.4	81.4	—1.2	—3.7	N.S.
Ascorbic acid	1.7	45.0	98.5	118.0	87.6	166.4	90.0	104.0	.01

[1] Almost significant at the .05 level.

The above results, coupled with those obtained when inhibitors were used, strongly suggest that laccase and phenolase are active in the root of the sugar beet.

The changes in respiratory rate, a decrease of 9.2 percent with glycolic acid and a decrease of 3.7 percent with lactic acid, lacked significance. The probable absence of glycolic acid oxidase from the root tissue was thus indicated.

The increase in oxygen consumption, 104.0 percent, consequent to the addition of ascorbic acid to the respiratory medium of the root dics could signify either ascorbic acid oxidase or laccase activity, or both (11). The lack of inhibition of respiration by 8-hydroxyquinoline threw doubt on the participation of ascorbic acid oxidase. To clarify this situation the effect of pH on the oxidation of ascorbic acid by root homogenate was determined. The results are given in Figure 1.

The optimum pH for the beet root enzyme system capable of oxidizing ascorbic acid was found to be 6.6. This strongly suggests laccase was responsible, since this enzyme has an optimum pH range of 6.2 to 6.8, depending on source and purity (11). Ascorbic acid oxidase has a pH optimum of approximately 5.6. No evidence of a secondary peak in the curve at this PH was obtained.

The spectrophotometric determination gave no evidence of cytochrome oxidase activity. The change in optical density at 550 Mμ was the same in the presence and absence of enzyme preparation, *viz.* 0.05 optical density units per 10 minutes.

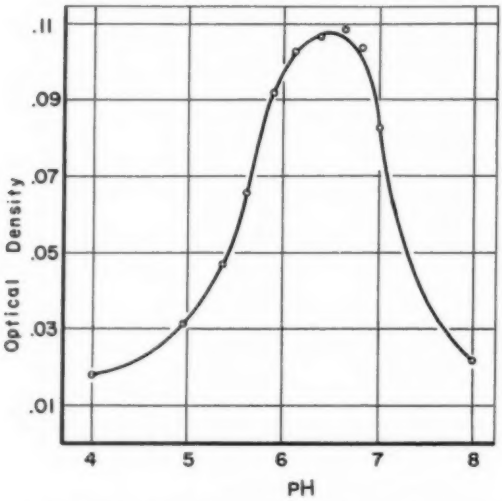

Figure 1.—Oxidation of ascorbic acid as affected by pH of sugar beet tissue homogenate. Optical density changes were followed at 265 mμ.

Discussion

The severe inhibition of oxygen uptake by sugar beet root discs which resulted from the inclusion of cyanide and azide in the respiratory medium strongly suggests that metallic oxidases are involved in the respiration of the tissue. In spite of the low level of inhibition obtained with thiourea, which may have resulted from lack of penetration of the tissue by this inhibitor, the marked inhibition produced by dieca indicates the activity of copper enzymes. While Honda (7) has shown that confusing results can be obtained with dieca, James (8) considers this chelating agent to be the most useful for distinguishing the copper enzymes from cytochrome oxidase in situ.

The inhibition obtained by the use of resorcinol, which acts primarily as a competitive inhibitor for phenolase (8), suggests that this enzyme may play a part in the respiratory process in root discs. The addition of catechol, a substrate of both laccase

and phenolase, resulted in a very considerable increase in respiration. Further evidence of the activity of these enzymes was furnished by the activation produced by p-phenylenediamine and hydroquinone, both of which are laccase substrates, and by tyrosine and protocatechuic acid, substrates of phenolase. The light stable inhibition of respiration by CO admits the possibility that phenolase may be present in the beet root tissue.

Ascorbic acid oxidase activity is questioned by the lack of response to the inhibitor 8-hydroxyquinoline, as Stout and Spikes (10) found, and to the absence of the characteristic ascorbic acid oxidase pH optimum in the oxidation of ascorbic acid by root homogenate. In this instance the optimum pH observed, pH 6.6, strongly suggests that laccase is the active oxidase.

In spite of the ubiquitous occurrence of cytochromes in plant tissue, as suggested by Smith and Chance (9), and others, the techniques used in this investigation failed to identify cytochrome oxidase as an active participant in the respiration of sugar beet root tissue. It has been emphasized by numerous writers that while light reversibility of CO inhibition of respiration shows the presence of cytochrome oxidase, the absence of light sensitivity in CO inhibited respiration is not conclusive evidence that an enzyme other than cytochrome oxidase is opertive (6, 9). The absence of cytochrome oxidase activity in beet root homogenate, as shown by the spectrophotometric technique, is difficult to explain away, however.

Summary

The results obtained by the use of numerous inhibitors and substrates of cytochrome oxidase, ascorbic acid oxidase, phenolase, laccase and glycolic acid oxidase; the absence of light-sensitive carbon monoxide inhibition; and the failure to detect cytochrome oxidase activity spectrophotometrically strongly suggest that, under the various conditions imposed by this investigation, the oxidases active in the respiration of mature sugar beet root tissue are laccase and phenolase.

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The Sodium-Potassium Nutrition of Sugar Beets Produced on Organic Soil[1]

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Introduction

The yield response of some crops to sodium has been known for nearly one hundred years. Voelcker as cited by Lehr (3)[3], obtained yield increases by treating mangels with sodium chloride as early as 1865. Harmer and Benne (2) were the first in Michigan to study the effect of salt applied to organic soil on the yield and chemical composition of crops. They reported in 1941, a six year average increase of 4.3 tons of sugar beets per acre from 500 pounds of sodium chloride (salt) where the average annual potash rate was 136 pounds per acre.

Information obtained in the forties at the newly developed Michigan Muck Experimental Farm and reported by Davis (1) in a summary statement show sugar beet yield increases of 1 to 1.8 tons from salt where the potash rate was 300 pounds per acre and no response from salt where the potash rate was doubled. Reports from growers have indicated inconsistent results from the application of salt to sugar beets and the data obtained over the years indicate the yield response from salt to be related to the level of soil potassium or the use of potash fertilizer.

This study was initiated on existing plots at the Muck Experimental Farm to determine the yield response of sugar beets to salt at various soil levels of potassium as measured by soil test and to determine yield response of sugar beets to salt when additional potassium was applied as fertilizer across soils varying in levels of potassium.

Procedure

An investigation involving the response of crops to salt at varying rates of potash was established on Houghton muck located at the Michigan Muck Experimental Farm in 1951. The soil had a pH of 6.3 and contained 85 percent organic matter as determined by loss on ignition. Potash was applied at four rates: 100, 200, 300, and 600 pounds per acre annually. Each block was split and to one-half, 500 pounds of salt were applied annually. Celery and table beets were grown on these plots from 1951 through 1954. Soil tests were taken in the spring of 1955. These tests indicated uniform levels of phosphorus

[1] Contribution from the Soil Science Department, Michigan Agricultural Experiment Station, East Lansing, Michigan, and authorized for publication by the Director as Journal Article No. 2401.

[2] Soil Science Department, Michigan Agricultural Experiment Station, East Lansing, Michigan.

[3] Numbers in parentheses refer to literature cited.

across all plots in the experiment and a uniform potassium level for each of the four rates of potash applied.

In 1955, the plots were split a second time. One-half of each plot received the regular application of potash while the other half was left unfertilized.

The plots were cropped with US 400 sugar beets for two consecutive years, 1955 and 1956. Soil samples were taken each fall and spring and tests for phosphorus and potassium were made by the Spurway Active method.[4] The planting dates were May 20, in 1955 and June 2, in 1956. The harvest dates were November 8, in 1955 and November 7, in 1956.

At harvest the fresh weights of the tops and roots were recorded and samples for chemical analysis were taken.[5]

Results

As shown by the data in Table 1, the yields of tops were fairly uniform across all treatments with no significant differences in weight apparent. Likewise, no significant differences in the percent of sucrose resulted from treatment. Potash did, however, cause significant increases in root yields. Yields from plots receiving 200 pounds of potash were significantly higher than those from the plots treated with 100 pounds. Also, the yields from the plots treated with 600 pounds of potash were significantly higher than those from plots treated at the 200 pound rate. The salt did not significantly increase yields on the fertilized plots, but the data indicate a yield response from the salt where the two lowest levels of potash were used.

Considering the plots which were not fertilized in 1955, significantly higher yields of sugar beets were obtained from the plots previously treated with the 600 pound rate of potash than from any other potash level. Also, the residual potassium from plots previously treated with 200 or 300 pounds of potash, produced yields of beets that were significantly higher than the yields from plots treated at the 100 pound rate.

Salt applied on the plots not fertilized in 1955 was as effective in increasing the yield of beets as was the 1955 potash treatment.

The data in Table 2 show that 1956 yields and sugar percentages were considerably different than those of 1955.

In 1956 the yields of roots from plots receiving the three highest rates of potash (200, 300, and 600) were significantly higher than the yields from plots receiving the 100 pound rate. Also, the plots where either 100 or 200 pounds of potash were

[4] .018 normal acetic acid extract.
[5] Sodium and potassium analyses supplied by E. J. Benne and the staff of the Agricultural Chemistry Department.

Table 1.—The Effect of Sodium Chloride at Varying Rates of Potash on the Yield and Sucrose Content of Sugar Beets, 1955.

Fertilizer Grade	Fertilized Annually[1]				Unfertilized 1955[2]			
	Tons per Acre			Percent Sucrose	Tons per Acre			Percent Sucrose
	Tops	Roots	X̄		Tops	Roots	X̄	
0-10-10 + Salt[3]	13.9	16.8	15.3	11.7	14.5	14.4	12.8	10.8
0-10-10	14.2	13.8		11.8	12.7	11.3		11.5
0-10-20 + Salt[3]	12.9	21.7	20.2	11.8	15.7	19.4	17.0	12.2
0-10-20	14.5	18.7		12.3	15.1	14.6		11.5
0-10-30 + Salt[3]	15.4	22.3	21.5	12.0	15.3	20.6	18.9	12.0
0-10-30	14.3	20.7		12.2	15.6	17.2		12.4
0-10-60 + Salt[3]	12.2	23.0	23.2	11.9	15.9	25.1	23.8	11.0
0-10-60	14.6	23.5		11.8	15.9	22.5		11.4
LSD (5% level)	N.S.		2.3	N.S.	N.S.		3.3	N.S.

[1] Fertilized from 1951 through 1955 at 1000 pounds per acre of grade indicated, 100 to 600 pounds K_2O.

[2] Fertilized from 1951 through 1954 at 1000 pounds per acre of grade indicated but not fertilized in 1955.

[3] 500 pounds of salt per acre (sodium chloride).

applied and which received salt, produced higher beet yields than did the corresponding plots which did not receive salt. By grouping the data from all plots which received potash, it was possible to show that salt caused significantly higher sucrose contents.

In 1956, the yield of beets from the fertilized plots was considerably higher than those unfertilized. The data from the unfertilized plots show that in every case sugar beet yields were increased by salt. These data indicate that a yield response of sugar beets to the application of salt may be related to potassium level in the soil or to the rate of potash applied, with response to salt decreasing with increased potassium level in the soil.

The data in Figure 1 show the potassium level of the soil in the spring of 1955 and the potassium level in the spring of 1957 following the two-years cropping with sugar beets. The loss of potassium from the soil unfertilized in 1955 and 1956 as a result of cropping is apparent. The highest soil test level in a two-year period dropped from about 300 pounds of potassium per acre to less than 50 pounds.

Table 2.—The Effect of Sodium Chloride at Varying Rates of Potash on the Yield and Sucrose Content of Sugar Beets, 1956.

Fertilizer Grade	Fertilized Annually[1]				Unfertilized 1955 and 1956			
	Tons per Acre			Percent[1] Sucrose	Tons per Acre			Percent Sucrose
	Tops	Roots	$\bar{X}$		Tops[2]	Roots[2]	$\bar{X}$	
0-10-10 + Salt[3]	13.1	10.6	9.9	14.9	13.5	8.6	7.2	14.0
0-10-10	10.8	9.2		14.1	8.2	5.8		14.1
0-10-20 + Salt[3]	13.3	12.2	11.6	15.3	13.0	8.0	7.1	13.9
0-10-20	13.3	11.1		15.2	9.1	6.3		14.3
0-10-30 + Salt[3]	11.8	11.8	11.7	16.5	11.0	8.7	8.0	14.5
0-10-30	13.4	11.5		15.1	11.1	7.4		14.4
0-10-30 + Salt[3]	11.1	11.8	12.1	16.0	11.4	9.9	8.7	13.9
0-10-60	12.5	12.5		14.4	10.0	7.6		13.6
LSD (5% level)	N.S.		1.7				1.7	N.S.

[1] Significantly higher sugar content in favor of salt treatment (5% level).
[2] Significantly higher root and top yields in favor of salt (5% level).
[3] 500 pounds of salt per acre (sodium chloride).

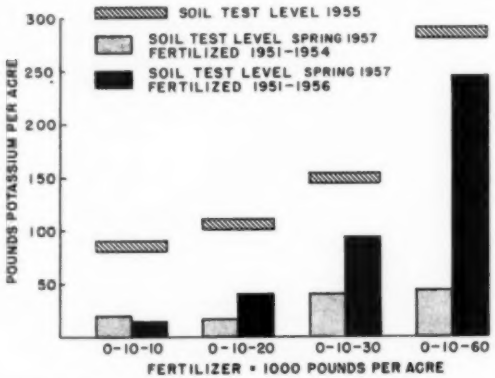

Figure 1.—The effect of two consecutive years of sugar beet cropping on the potassium level in the soil.

In 1955, with little difference in sugar content, the sugar produced per acre paralleled yield (Figure 2), thus giving higher sugar yield as a result of salt applied within the normal range of potash fertilization. In 1956, with little difference in

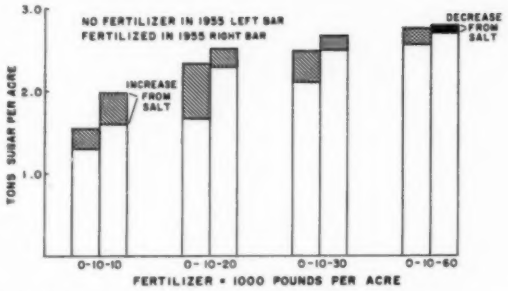

Figure 2.—The effect of sodium chloride and potash on the yield of sugar, 1955.

yield on the fertilized plots but a higher sugar content in beets from the salt treated plots, higher yields of sugar per acre were produced where salt was applied.

The potassium and sodium content of the sugar beet roots and tops are given in Tables 3 and 4. The sodium and potassium contents of sugar beet tops were considerably higher than the roots. In general there appears to be an inverse relationship between the sodium and potassium content of the tissue. The tissue with the highest content of potassium, in general contained the lowest content of sodium, and vice versa. Due to higher yields of beets where salt was applied, in general more potassium was removed per acre from plots receiving salt than from corresponding plots receiving no salt, even though the

Table 3.—The Effect of Sodium Chloride at Varying Rates of Potash on the Potassium and Sodium Composition[1] of Sugar Beet Roots, 1955.

Fertilizer Grade	Fertilized Percent		Unfertilized Percent	
	Potassium	Sodium	Potassium	Sodium
0-10-10 + Salt[2]	1.01	.90	.98	1.06
0-10-10	1.21	.39	.79	.76
0-10-20 + Salt[2]	2.22	.63	.91	2.10
0-10-20	1.55	.44	1.49	.16
0-10-30 + Salt[2]	2.23	.60	2.27	1.28
0-10-30	2.34	.38	2.28	.19
0-10-60 + Salt[2]	2.75	.31	2.45	.65
0-10-60	2.97	.24	3.44	.53

[1] Chemical composition expressed at percent of the oven dry tissue.
[2] 500 pounds of sodium chloride per acre.

Table 4.—The Effect of Sodium Chloride at Varying Rates of Potash on the Potassium and Sodium Composition of Sugar Beet Tops,[1] 1955.

Fertilizer Grade	Fertilized Percent		Unfertilized Percent	
	Potassium	Sodium	Potassium	Sodium
0-10-10 + Salt[2]	2.28	3.09	4.04	2.25
0-10-10	3.20	1.76	2.87	3.07
0-10-20 + Salt[2]	5.16	2.12	3.81	2.80
0-10-20	4.72	1.62	2.91	2.61
0-10-30 + Salt[2]	5.01	2.19	4.03	2.03
0-10-30	5.39	1.32	4.06	.98
0-10-60 + Salt[2]	6.35	1.97	5.73	2.69
0-10-60	6.49	1.01	4.77	1.88

[1] Chemical composition expressed as percent of oven dry tissue.
[2] 500 pounds of sodium chloride per acre.

content of the tissue may have been slightly less. This is especially true within the range of normal potash fertilization, (200 and 300 pounds).

Summary

Tests conducted at the Michigan Muck Experimental Farm show that the yield response of sugar beets to salt applications decreased with increased rates of potash applied. Salt applied at 500 pounds per acre across plots receiving 600 pounds of potash per acre tended to depress the yield of sugar beets.

The data indicate an inverse relationship between the potassium and sodium contents of the sugar beet tissue in that high levels of potassium tended to reduce the sodium content, and vice versa. Even though this relationship existed, more potassium was removed per acre in the crop from salt treated plots at the 200 and 300 pound per acre rates of potash.

The large loss of potassium as indicated by soil test indicates the need for annual soil tests to determine fertilization recommendations for sugar beets.

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A Procedure for Squash Preparations of Somatic Sugar Beet Tissues

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In the routine cytological examination of somatic tissues of sugar beets, a need was felt for a procedure that would give better preparations, require less handling time, and allow considerable latitude in time intervals. Attempts were made to improve the procedure and the quality of the preparations. The procedure described herein was satisfactory. It has only four steps: shrinking the chromosomes; killing, fixing, and macerating the tissues; washing out the killing, fixing, and macerating fluid; and squashing the tissue in the stain. The method is different from other methods in that the killing and fixing step is combined with the macerating step.

Materials

Tjio and Levan (1)[2] first suggested the use of 8-hydroxyquinoline as a shrinking agent. For the procedure described herein, a .002 M. (290 mg. per L.) solution of 8-hydroxyquinoline was prepared by dissolving the crystals in distilled water at 60° C. Vigorous stirring for 10 to 15 minutes brought most of the crystals into solution.

The killing, fixing, and macerating solution was a mixture of 7 parts Newcomer's fluid (2) and 3 parts 1 N. HCl. (Newcomer's killing and fixing solution is composed of 6 parts isopropyl alcohol, 3 parts propionic acid, 1 part acetone, 1 part dioxane, and 1 part petroleum ether.) For best results, the Newcomer's fluid and the acid should be mixed immediately before using.

Von Rosen (3) recommended spirit soluble nigrosin for staining beet chromosomes. The junior author of the present paper has tested several sources of nigrosin, some of which stained the chromosomes only lightly or not at all. Among the sources, however, two were found to be outstanding. The first was "Spirit Nigrosin No. 19305," made by W. S. Simpson & Co., Ltd., Old Southgate, London, N. 14, and supplied by Sidney Ellerton.[3] Dr. Ellerton also furnished information on his method for preparation of the stain, which is as follows:

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[1] Geneticist, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland; and Assistant Plant Breeder, Danish Sugar Company, Sophieholt, Rodby, Denmark, respectively.

[2] Numbers in parentheses refer to literature cited.

"Take 50 cc. glacial acetic acid and 50 cc. distilled H_2O. Bring to boil and add 4 gm. of nigrosin gradually while stirring. Boil gently for 5 minutes. Cool rapidly. Filter. Dilute 1 part filtrate with 2 parts 45% acetic acid."

The second source of spirit soluble nigrosin was Arthur H. Thomas Co., Philadelphia, Pennsylvania. No appreciable difference between the two stains was noticed. In addition to Dr. Ellerton's method, the stains were also prepared by two other, slightly different methods of heating. In one method the solution was boiled in a refluxing condenser for ½ to 1 hour. In the other method the solution was placed in an autoclave for 20 minutes at 15 pounds pressure. No great differences in stains heated by the three methods were noticed.

The senior author has also had satisfactory results with synthetic orcein. One and one-half grams of dye were placed in a flask with 50 ml. of 60 percent acetic acid. The flask was placed in an autoclave at 15 pounds pressure for 20 minutes. The stain was then cooled and filtered. Ten drops of Aerosol OT (dioctyl sodium sulfosuccinate) were added. The formation of precipitate made it necessary to filter the stain at weekly or biweekly intervals.

Methods

Either leaves or root tips could be utilized in the procedure described here. When leaves were used, one of the smallest from the growing point, which was usually 3 to 8 mm. long, was taken; when roots were used, only snow-white root tips were taken. The leaves or root tips were placed in small vials with 8-hydroxyquinoline solution for 3½ to 4 hours at 55° to 65° F. The 8-hydroxyquinoline was poured out, and the killing and macerating fluid was added. The plant material was left in the fluid for ½ to 3 hours, after which the killing and macerating fluid was replaced with water. Squashes were made after the tissue had been in the water for 10 minutes to 24 hours. It was possible to make counts on tissues stored in water in the refrigerator for a week. About 1 mm. of the root tip or ½ mm. of the basal part of the beet leaf was used in making the squash. It was placed in a drop of stain on a slide, and a cover glass was put on top. Pressure applied to the cover glass spread the tissues. A slight lifting of one edge of the cover glass allowed the stain to run under and come into more intimate contact with the

[8] Dr. S. Ellerton, British Pedigree Beet Seed, Ltd., Maldon, Essex, England, tested several sources of nigrosin, found this one best, and kindly supplied samples to workers in the United States.

cells. After 30 seconds (or a few minutes if darker staining was desired), pressure was again applied to the cover glass to flatten the cells.

Discussion and Results

The greatest advantage of the procedure is the simplification of the maceration process by combining it with the killing and fixing process. Since the maceration was completed by the time the material was fixed, the time required for the preparation of each slide was reduced to a minimum. The maceration allows the cells to be spread apart and flattened rather readily without disintegration of the cell walls. The considerable time latitude for each of the steps of the process allowed the technician to use a rather flexible schedule. The flexibility, however, had certain limitations. Keeping tissues in the 8-hydroxyquinoline solution less than 3 hours resulted in preparations with the chromosomes too long and not well spread in the cell. Keeping tissues in the solution longer than 4½ hours gave preparations with few mitotic divisions and with stained cytoplasm. Increased amounts of 1 N. HCl in the killing solution resulted in less stain being absorbed by the chromosomes, while less HCl resulted in inadequate separation and inadequate flattening of cells. After 3 or 4 days in water, the chromosomes became more elongated and were not as suitable for observations.

Excellent preparations were obtained from about 90 percent of the beet plants examined by this method. Failure to obtain good preparations could usually be attributed to an unthrifty condition of the plant.

Summary

A new cytological procedure for the preparation of sugar-beet somatic tissues was developed. The outstanding feature of the procedure was combining the macerating process with the killing and fixing process.

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Pollen Sterility in Beta Vulgaris Associated with Red Pigmentation of the Anthers[1]

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The anthers of most plant species are yellow at time of dehiscence. However, many species exhibit pigmented anthers. The color of these anthers varies as greatly as the pigmentation found in other plant parts. This paper deals with a relationship between red pigmentation of the anthers and pollen sterility in table beets.

Materials and Methods

The plant materials used in this study were either Perfected Detroit with a genotype assumed to be RRYY for pigmentation, sugar beet material assumed to be the genotype rryy or rrY^rY^r, or progeny resulting from the crosses between these two types. The studies were conducted in materials carrying the cytoplasmic factor for pollen sterility and materials carrying the normal cytoplasmic factor.

Observations and Results

In 1954 a plant of Perfected Detroit was found in which about five percent of the anthers was a very bright red. These anthers were devoid of functional pollen. The remainder of the anthers on this plant were yellow and normal-appearing pollen dehisced freely from each. From subsequent analysis it was found that each of the pigmented plants growing in the greenhouse, most of which were of the variety Perfected Detroit, had some red anthers. The frequency varied from a few per plant to as many as 30 percent. In all cases the red pigmented anthers were sterile or partially sterile. A few were found that had a maximum of two percent pollen fertility based on staining and microscopic examination. In some plants there was an intermediate type of pigmentation in which the anthers were spotted, part of the anther being yellow and part being red. In these anthers the fertility was much reduced but seldom were the anthers devoid of some functional appearing pollen.

The colored anthers did not appear in any particular pattern on the plant. A detailed analysis was made of the location of the anthers and their pigmentation. Although the data were

[1] Published with the approval of the Director of the Agricultural Experiment Station. This investigation was supported in part by a grant from the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation and is a portion of a thesis presented to the faculty of the University of Wisconsin, August, 1957, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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not completely random they were nearly so, and it can be assumed that the red anthers did not develop in distinct sectoral patterns.

Most of the yellow anthers on the plants were highly fertile (Table 1). The range of fertility among the yellow anthers was distinctly higher than the range of fertility for spotted anthers. Similarly, the red anthers and the spotted anthers were of two distinctly different ranges in fertility. Occasionally spotted anthers were found in which none, or practically none, of the pollen grains appeared viable.

The difference between red anthers and spotted anthers suggests a quantitive effect associated with the amount of anther which is pigmented. Table 2 presents a summary of data show-

Table 1.—The Association Between Anther Color and Sterility.

Anther Color	Average Fertility (%)	Range of Fertility (%)	Number of Plants
Yellow	80.62	51.68-96.97	18
Spotted	11.11	0—39.68	15
Red	0.50	0— 1.50	11

Table 2.—The Relationship Between Size of Red Spot and Pollen Sterility.

Plant Number	Origin of Line	Anther Color	% Fertile	Significance
205-4	Early	Yellow	81.22	**
	Blood	Small spot	14.63	**
	Turnip	Med. size spot	2.08	
21-5	Detroit	Yellow	a	
	Dark	Small spot	75.53	**
	Red	Large spot	24.19	**
		Red	0.00	**
124-10	Early	Yellow	a	**
	Wonder	Small spot	33.53	**
		Med. size spot	0.00	**
62-5	Perfected	Yellow	a	
	Detroit	Med. size spot	0.70	**

a Inspection of anther smears indicated normal pollen development (above 95 percent fertile).

** Significant at 1 percent level or by inspection.

ing the relationship between the size of the spots and the degree of fertility. In these cases anthers were judged either as having a small, a medium or a large sized spot. Considerably less than half of the anther was pigmented in a medium sized spot. Since these comparisons were made between spotted and yellow anthers on the same plant it is assumed that gross genetic differences have been eliminated. These data support the hypothesis that the degree of sterility is associated in some way with the amount of pigmentation in the anther.

The Dependence of Pigmentation on Genotype

Red anthers were not found on sugar beets. Therefore, F_2 populations from the cross "red beet x sugar beet" were evaluated to determine if the presence of red anthers was dependent on a genotype which controlled the red pigmentation in the beet root. Each of the red pigmented F_2 roots produced red anthers. However, 5.61 percent of the non-pigmented had red anthers, the remainder of the non-pigmented plants being devoid of red anthers in a manner similar to the sugar beets. Since these plants were flowering when classified, it is possible that the 5.61 represented plants of the genotype Ry which would be expected as crossover genotypes in 3.84 percent of the cases. Ry plants would be expected to have green tops, pink hypocotyls and white roots (2)[3]. These data suggest the possibility of a dependence for the gene R if red anthers are to be expressed.

The Dependence of Pigmentation on Substrate

Anthocyanin accumulates in many plants at sites where injury has occurred or where the orderly translocation of the materials to or from a region is impaired. Since these anthers arose quite randomly on the plant, it was possible that such a mechanism was operative here. Two types of experiments were conducted to test the possibility that substrate influenced the degree of sterility and/or the degree of pigmentation.

In the first experiment the tips of flowering stalks were immersed in a sugar solution. Of thirteen plants treated, five flowering stalks that were immersed changed color noticeably and associated with this change in color was the obvious increase in the percent of red anthers. In all five plants exhibiting this color change, the treated branches were significantly higher in percent red anthers than the untreated branches. The percent of red anthers was higher near the point of immersion than at the base of the same branch. In the inflorescences which failed to change color there was no apparent increase or decrease in

[3] Numbers in parentheses refer to literature cited.

Table 3.—The Effect of Immersion in Sugar Solution on the Frequency of Red Anthers in the Treated and Untreated Branches of Five Selected Plants.

Plant No.	Percent Red Anthers	
	Treated	Untreated
123-3	100.0	25.8
123-8	64.2	21.0
127-3	69.1	10.3
129-1	25.5	1.0
200	82.8	10.8
Mean	68.3	13.8

the percent of red anthers. Table 3 lists the five plants which responded positively to the treatment. In no case did plants have fewer red anthers following treatments than before.

The five plants listed in Table 3 probably represented plants in which adequate amounts of sugar entered the plant and thereby influenced the amount of anthocyanin and/or sterility in the plant. In all cases the red anthers were pollen sterile and the yellow anthers were pollen fertile on these plants. The data are significant by inspection.

In another study anthers were removed from the plant prior to dehiscence and/or pollen break-down and grown *in vitro*. A liquid coconut culture medium containing two percent sucrose was used. One anther from each flower was fixed in FAA to use as a control for size of anther and stage of development at the same time the other four anthers were placed on the growth medium. It was impossible to grow the anthers to complete maturity *in vitro*. However, it was possible to detect noticeable differences in rates of growth and stage of final development between red anthers and yellow anthers. In nearly all cases the percent of red anthers was greater *in vitro* than the percent of red anthers on the plant from which the anthers were removed. In no case were there fewer red anthers *in vitro* than *in vivo*. The anthers which remained yellow *in vitro* grew larger than those that showed any red pigmentation (Table 4). Similarly the spotted anthers grew more than those that turned red but less than those that turned yellow.

The stage of final growth of the pollen grains is shown in Table 5. Stage A in Table 5 represents a stage somewhat earlier than stage B. Stage A can be described as follows: The microspores have been released from the tetrads and have changed

Table 4.—Increase in Size of Yellow and Colored Anthers *in Vitro* Cultures (Measured in Uncalibrated Micrometer Units).

Anther Color	Average Anther Growth	Number of Observations
Yellow	2.85	181
Spotted	2.53	62
Red	2.28	45

Table 5.—The Relationship Between Anther Color and the Percentage of Anthers in the Advanced Stages of Development *in Vitro* Cultures.

	Yellow		Spotted		Red	
	Stage A	Stage B	Stage A	Stage B	Stage A	Stage B
Percentage of all anthers	20.2	30.1	40.7	11.1	23.3	3.3
Total	50.3		51.8		26.6	

their shape from polyhedral to spherical. The exine has hardened and the characteristic buckles have appeared. The cells do not stain with acetocarmin. This failure to stain is probably associated in some way with the hardening of the exine. Stage B, which is a more advanced stage, is a continuation of stage A with very prominent buckles. The pollen grains assume a light pink hue with acetocarmin.

The percent of anthers in these final two stages of pollen development in the red anthers was only about half as great as in the spotted or yellow anthers, with no difference between the latter two. However, if the comparison is made between the more mature stage B and the somewhat earlier stage A it is obvious that the yellow anthers proceeded to stage B in a greater porportion of the time. It would appear that in spotted and yellow anthers approximately 50% reached stage A, but that a much higher proportion of these anthers were arrested in their development at stage A in the spotted anthers than in the yellow anthers. These data substantiate the concept that pigmentation is important to the expression of growth and sterility. The increase in pigmented anthers on this energy rich medium over *in vivo* anthers substantiates the data in Table 3. In no case did red anthers appear in cultures grown from unpigmented plants.

The Pigmentation of Anthers in Pollen Sterile Cytoplasm (S).

In a number of breeding lines of table beets the cytoplasmic factor for pollen sterility had been introduced previously from sugar beets by a continuous backcrossing program to table beets. It was noticed that the sterile plants in these lines had an extremely high proportion of pigmented anthers. This was in contrast to those plants studied previously which had normal cytoplasm and the percent of red anthers varied from very few to about 30 percent. Nearly 100 percent of the anthers on many of the sterile plants in these cytosterile lines were red. An obvious implication would be that the sterility, brought about through the action of the cytoplasmic factor and the appropriate related genes (S xx zz) (1), was enhancing and causing the anthers to be pigmented. However, in certain cytosterile red table beets many plants are also largely devoid of red anthers. This would seem to preclude the possibility that the sterility caused the pigmentation in these anthers.

Discussion

The red pigmentation in anthers of beets is very closely associated with a pollen-sterile condition. The evidence obtained in these studies suggests a dependence upon at least one of the basic color genes which is common to red beets but not to sugar beets.

It is difficult from the studies completed thus far to differentiate cause and effect. The increased number of red anthers in cytosterile plants would suggest that sterility perhaps causes or at least enhances the red pigmentation. It is possible that other secondary effects are brought about in the substrate of the maternal tissue when the microspores abort, which would leave an energy rich medium available for synthesis of the pigment.

It is somewhat more logical to assume that red pigmentation, regardless of its cause, will bring about pollen sterility in anthers which are genetically pollen fertile. This was supported by at least two sources of evidence which suggest that by increasing the sugar in the substrate either *in vivo* or *in vitro* there is a marked increase in the percent of red anthers.

Similarly, it is difficult to visualize a mechanism by which sterility would take place in the anthers in red table beets, but not necessarily in non-pigmented beets, unless the pigmentation were the causal agent or closely related thereto. If the mechanism worked similarly in both, similar proportion of sterile anthers would be expected in the unpigmented and the pigmented

beets. This was not the case. If, however, the sterility could be brought about only in the presence of the large gene R, it would follow that whatever shifts in substrate that enhances the development of anthocyanin in the anthers would also cause pollen sterility. This is borne out by the observation that some anthers have only a red spot and produce only partially sterile anthers and others are entirely red and entirely sterile.

Conclusions

1. A correlated response between anther pigmentation and pollen sterility has been noted in table beets.

2. The percent of red anthers on pollen fertile plants (normal cytoplasm) ranged from very few to about 30 percent, with all pigmented plants showing at least a few anthers pigmented. In the cytosterile plants the proportion of red anthers was much greater.

3. All pigmented plants of F_2 populations segregating for pigmentation produced red anthers. However, 5.81 percent of the non-pigmented plants also produced some red anthers.

4. In no case was similar pollen sterility found in non-pigmented sugar beets used in these studies.

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Reversion in Cytoplasmic Male Sterile Plants of Beta Vulgaris[1]

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Cytoplasmically inherited pollen sterility has been reported in an ever-increasing number of plant species over the past thirty years. Its utilization in the production of hybrid seed has increased similarly. Yet the carriers of cytosterility are still unknown. Two hypotheses dealing with the nature of the carrier of cytosterility have received special attention. According to the first, the determinants are particulate units, which are not transmissable from cell to cell except by division (2)[3]. The determination of cytoplasmic characters, such as "killer" in *Paramecium* (5) and certain types of chlorophyll variegation in *Epilobium* (3) have been ascribed to particulate, non-genic factors, too. According to the second hypothesis, cytosterility is determined by a non-particulate, diffusible substance, not necessarily synthesized at the site of its action. In *Petunia*, cytosterility has been reported to be transmitted through a graft (1). The observations on reversion of male sterile beet plants to partial fertility reported in this paper support the second hypothesis.

Materials and Methods

Cytosterile lines of sugar beets were received from Dr. F. V. Owen in 1949. Cytosterile lines of table beets were obtained by systematically backcrossing these steriles and their resulting progenies to pigmented table beets. Within these backcrossed progenies were many lines segregating for sterility and fertility. It was assumed, based on Owen's hypothesis (4), that these steriles were of the genotype S xx zz. The original source of cytosterility was maintained as a sterile non-pigmented line by backcrossing continuously to the non-restorer line SLC 03. These latter steriles, hereafter referred to as "original cytosteriles," were used as controls to study the behavior of certain of the "derived cytosteriles" which were obtained in the table beet material.

The entire plant population studied was subjected to long days and low temperatures while seedlings and was thereby induced to flower in the first year. Because of this forced development, vegetative growth prior to flowering was somewhat restricted.

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[3] Numbers in parentheses refer to literature cited.

Observations

Among the derived cytosteriles were numerous red plants which also had bright red anthers (6). Pollen of these derived pigmented cytosteriles did not differ in development from pollen of unpigmented cytosteriles. The endothecium was unspecialized. The microspores disintegrated soon after being released from tetrads; their surface was soft and undifferentiated.

In many of these pigmented plants, anthers of late-appearing flowers underwent a conspicuous change. These were the flowers at the tip of the main flowering stalk and on the late flowering lateral stalks. The red color in the anthers disappeared, the anthers turned yellow and opaque, became more plump, and resembled semi-sterile anthers in appearance. A similar change in anther appearance was subsequently found in plants devoid of red pigment, but the color contrast between old and young anthers was not as conspicuous. Microscopic examination revealed that in these changed anthers the typical spiral thickenings of the endothecium were present, as well as xylem vessels. These anthers and their pollen grains resembled the semi-sterile plants reported by Owen to carry S cytoplasm and one or two of the restorer genes.

The pollen of reverted anthers was always more completely developed than pollen of earlier developed red anthers and resembled the semi-sterile pollen in possessing a sculptured exine. Prior to reversion, microspores did not persist long after release from tetrads, and anthers were typically flabby and semi-transparent. At the onset of reversion, in flowers close to the tip of the flowering stalk, pollen grains developed to a more advanced stage. A soft exine began to appear, but maturity was not reached. In still later appearing flowers, well-formed pollen grains of very regular spherical shape developed with a hard exine covered with prominent buckles. These pollen grains resembled those found in normal plants immediately prior to anthesis. At this stage, the anthers were opaque and yellowish. At a still later stage, a varying percentage of fertile pollen grains was found. Except for this simultaneous occurrence of fertiles and steriles in the last stage, pollen grain samples, as obtained from any single anther population were very uniform at any given moment, and the change in pollen development was gradual. The change in anther appearance and in the somatic anther tissue sometimes preceded the shift in pollen development. Reversion apparently affected the somatic tissues directly and somewhat earlier than the gametic tissues.

The plants, as grown in the greenhouse, differed somewhat in flowering time. While some plants were at or past the peak of flowering, others bore immature flowers in the inflorescence. Even though all plants were subjected, as nearly as possible, to the same environmental conditions, reversion never occurred in plants in which flowering had been delayed, or in which flowering was just starting, but occurred only in plants which had flowered for a considerable length of time. This seems to indicate that reversion is not brought about by specific environmental factors, but the internal condition of the plant itself. Reversion occurred in plants which were flowered particularly early. These plants had considerably less vegetative mass at flowering time than plants which did not revert.

Reversion was never observed in the "original sterile" lines. In only one case was a change in the endothecium noted. This line was maintained by back-crossing to the normal non-restorer SLC 03, hence it may be concluded that SLC 03 does not transmit the ability or tendency to revert to its offspring. Most of the plants in which reversion occurred were in F_2 populations of the cross (4460h0 x SLC 03) x Perfected Detroit. Since the seed parent of this original cross did not revert, it is assumed that the reversion occurred under the influence of the Perfected Detroit genome. This would not exclude genetic recombination within these lines.

After reversion had been observed in cytosteriles, it was sought in semi-steriles, too. The percentage of fertile grains was expected to be higher in late appearing flowers, at the upper part of the flowering stalk. A single plant was chosen for this study; since all anthers on the plant were uniform in appearance, they were chosen by location only. Anthers from the upper part of the inflorescence had an average of 34 percent fertile appearing pollen grains, while anthers from the lower part of the inflorescence averaged 20 percent fertile appearing pollen grains. This difference was highly significant.

Discussion

Reversion of pollen sterile plants to pollen fertile plants has been noted by several workers, and the cause has usually been ascribed to environmental factors. The present observations reported here indicate, however, that the reversion of plants under nearly identical environmental conditions depends on some internal factor or factors. Reversion of the cytosterile plants to partial fertility occurred in those plants in which the vegetative-

generative balance had been shifted toward the generative side very early. The vegetative development of these reverted plants had been interrupted by early flowering. This relationship suggests that the vegetative plant parts produce some substance or substances which are necessary for the maintenance of the sterile state. Reversion appears to be dependent on the exhaustion of reserve substances which accumulated during vegetative growth. These hypothetical substances could not be cell limited, as they appear to be transmitted to several parts of the plant simultaneously.

These results do not preclude the possibility that reversion could be brought about by either an exhaustion of a "sterility substance" or by accumulation of "fertility substances" in certain cells through random segregation until a certain threshold is reached. Neither do they preclude the exhaustion or accumulation of a diffusible substance or substances which could activate or deactivate cell limited particulate units.

The cell-limited particulate hypothesis implies that reversion would be associated with a particular cell lineage, in which random segregation has led, by chance, to an accumulation of fertile particles or depletion of sterility particles. It does not explain the simultaneous occurrence of reversion at different sites on a plant, as observed in the beet. However, the simultaneous effect in different plant parts does fit the assumption of a diffusible substance. The association of reversion with changes in somatic anther tissue, and the concommitant changes in pollen development are also more easily explained by the assumption of control by a diffusible substance.

The reversion that takes place from "derived cytosteriles" to conditions of partial fertility is very similar morphologically to the differences that exist between the genotypes S xx zz and S Xx zz, or S xx Zz, or S Xz Zz. This implies that within the plant physiological changes can take place of the same magnitude as known genic changes.

Although reversion has been observed in "derived cytosteriles" only, the mechanization probably has broader application pertinent to the nature of the cytoplasmic factor and thereby be applicable to cytosterility in general. The data also suggest possible mechanisms for the instability of certain steriles under different cultural and environmental conditions.

Summary

Cytoplasmic male sterile sugar beets were crossed by fertile table beets. Some segregants of these crosses started out as typical cytosteriles, but in their latest developed flowers, produced a varying percentage of fertile pollen (3%-19%). This reversion was not caused by environmental factors alone, but depended on some internal condition of the plant, related to the relationship between vegetative and generative development. An early shift of the normal balance toward generative development is associated with reversion. This indicates, that the maintenance of the sterile state requires some product of the vegetative growth, probably a diffusible material.

Reversion is gradual, and different stages of the process can be distinguished. Pollen grain populations in a plant at any given moment are very uniform. This, too, supports the assumption that reversion is due to an accumulation of a "fertility substance" or to exhaustion of a "sterility substance": not to segregation of particulate sterility factors.

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Glutamic Acid Content of Sugar Beets as Influenced by Soil Moisture, Nitrogen Fertilization, Variety and Harvest Date[1]

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More effective use of the sugar beet and its byproducts will add needed stability to the sugar beet industry. During this time of acreage allotments, it is even more important that sugar beet processing be carried out in the most efficient manner.

The non-sugar constituents in the beet have been largely responsible for failure to extract all of the sugar (1)[3]. As an aid to solving this problem, there is need for a better understanding of the chemical constituents of the sugar beet and the effects of various physiological factors upon them.

One of the non-sugar constituents of the sugar beet which has received attention recently is glutamic acid. Preliminary investigations at the Utah Agricultural Experiment Station[4] showed that of the eight chemical constituents determined glutamic acid was the most variable. These results are in harmony with earlier work in the field (2) (4).

Derivatives of glutamic acid have considerable commercial value as food seasoners. A new industry has developed to manufacture and market these food seasoners, using the sugar beet as the main source of glutamic acid. The industry uses more than 100 tons of beet molasses daily (5).

Problems associated with glutamic acid in sugar extraction, and the value of the acid as a byproduct, prompted this study of the effects of some of the major agronomic factors upon the glutamic acid content of the sugar beet. The effects of these factors on root yields will be presented in a later paper.

Experimental Procedure

The effect of soil moisture, nitrogen fertilization, variety, and harvest date on the glutamic acid content of sugar beets was studied in a field experiment at Logan, Utah, in 1955. The

[1] Contribution from Agronomy Department, Utah Agricultural Experiment Station, Logan, Utah. Journal Paper No. 92. Part of a thesis submitted by Mr. Woolley in partial fulfillment of the requirements of an M.S. degree at Utah State University. The work carried out in cooperation with Western Utilization and Research Division, ARS.

[2] Former graduate student and Associate Professor of Agronomy, Utah Agricultural Experiment Station, Logan, Utah, respectively. The authors are indebted to D. A. Greenwood, J. L. Haddock, Bliss Crandall, and Rex L. Hurst for their assistance in planning and conducting the experiments.

[3] Numbers in parentheses refer to literature cited.

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design was a 3x3x2x2 split-plot factorial with moisture levels and varieties on the whole plots (30′ x 32′) and nitrogen levels on the sub-plots (10′ x 32′). Each sub-plot was divided into two sub-sub-plots (10′ x 16′) for use in measuring the effect of harvest date. The treatments were replicated four times. The plots were seeded with Planet Jr. hand seeders on April 30, using a uniform seeding rate of 15 pounds per acre. The rows were 20 inches apart, making 18 rows in each whole plot and 6 rows in each sub-plot. The plants were hand-thinned to an in-row spacing of 12 inches on June 8.

The moisture treatments were as follows:

M_0—Low moisture—irrigated when the moisture content of the root zone reached 25 ± 10 percent available water (about four atmospheres tension).

M_1—Medium moisture—irrigated when the moisture content of the root zone reached 50 ± 10 percent available water (about 1.5 atmospheres tension).

M_2—High moisture—irrigated when the moisture content of the root zone reached 80 ± 10 percent available water (about 0.5 atmospheres tension).

Bouyoucos blocs and tensiometers were used to measure the soil moisture tension. The bouyoucos blocks were installed at depths of 6, 12, 18, 24, and 48 inches on all moisture plots. Tensiometers were installed at the six- and twelve-inch depths on the M_2 plots only.

During the months of May and June the root zone was considered to be the six-inch depth; during July, the 12-inch depth; August, the 18-inch depth; and throughout September it was considered to be the 24-inch depth. As the season progressed the averages of the moisture tensions throughout the root zone were used to determine the time of irrigation.

Parshall flumes were used to measure the amount of water flowing on to the plots and also the amount of surface runoff. Irrigating was done by the furrow method. The soil moisture level was brought to field capacity with each irrigation. The amounts of irrigation water and natural precipitation received by the plots are shown in Table 1.

The three nitrogen levels were:

N_0—No nitrogen

N_1—80 pounds of elemental nitrogen per acre, applied at planting time in bands approximately 2 inches deep and 3 inches from each side of each row.

N_2—250 pounds of elemental nitrogen per acre, one-half side-

Table 1.—Amounts of Irrigation Water and Natural Precipitation Received by Sugar Beet Plots. Logan, Utah, 1955.

Treatment	Total Irrigation Water Applied Inches	No. of Application	Natural Precipitation Inches	
			Early Harvest	Late Harvest
			10/8/55	11/11/55
M_2—Moist	34	15	5.69	6.83
M_1—Medium	30	7	5.69	6.83
M_0—Dry	27	5	5.69	6.83

dressed near planting time and one-half side-dressed at mid-point in growing season (July 20).

All of the nitrogen was applied in the form of ammonium sulfate in bands approximately two-inches deep and three inches from each side of each row. All of the plots received 100 pounds of P_2O_5 per acre. This was broadcast on the surface of the soil and worked into the seedbed just prior to seeding.

The two varieties of sugar beets used in the experiment were US 22 Improved (SL 028), and Stewart's 53104-0[3]. The latter is the result of numerous selections from crosses between US 216, US 12, and US 22.

The two harvest dates were early (October 8, 1955) and late (November 11, 1955). At harvest the beets up to two feet from each end of each plot were discarded. To eliminate the effect of fertilizer and moisture from adjacent plots, only the center four rows of each sub-plot were sampled. At each harvest two samples, each containing 15 beets, were taken at random (avoiding the skips) from each plot (4 rows, 14 feet long). When the beets were dug they were immediately placed in ice water and held at 0° C. while they were transported to the laboratory. At the laboratory each 15-beet sample was taken from the ice water, thoroughly cleaned with cold water and bristle brushes, and weighed to the nearest one-tenth pound. Each sample was then placed in a Hobart food cutter (model T215) and chopped for one minute. This coarsely ground composite of each 15-beet sample was thoroughly mixed and a two-pound sample was returned to the chopper for an additional two minutes. This process produced a fine pulp of macerated material with no expressed juices. Several 10-gm. aliquots were taken from each sample for chemical analysis.

The glutamic acid content was determined microbiologically on a hot water extract using a modification of the method of Schweigert et al. (7).

[3] Obtained from Dewey Stewart, Sugar Beet Section, U. S. Department of Agriculture, Agricultural Research Service, Beltsville, Maryland.

Results and Discussions

Nitrogen fertilizer influenced the glutamic acid content of sugar beets in a highly significant manner (Figure 1). The relationship was almost linear. Each increase in nitrogen fertility increased the glutamic acid content of both varieties at both harvest dates. Beets which received nitrogen ranged from 0.019 to 0.216 percent glutamic acid and those without nitrogen ranged from 0.009 to 0.061 percent.

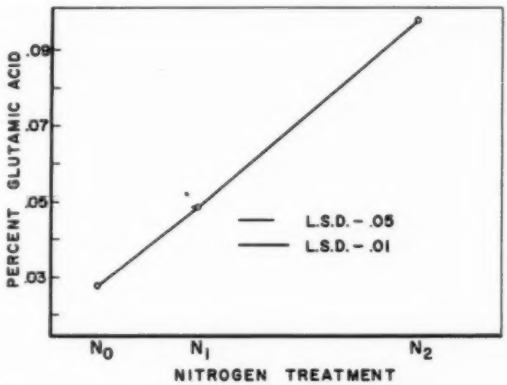

Figure 1.—Influence of nitrogen fertilization on the glutamic acid content of sugar beets, Logan, Utah, 1955 (Percentages given are averages over both varieties and both harvest dates).

The effects of the interaction between nitrogen and soil moisture on glutamic acid content are shown graphically in Figure 2. The data show that increased nitrogen applications up to 250 pounds per acre increased the glutamic acid content at each moisture level. The greatest moisture effect was observed at the high nitrogen level.

Hac et al. (3) found that the glutamic acid content of sugar beets increased progressively as the application of nitrogen increased up to 2500 pounds per acre.

The influence of moisture on glutamic acid content is shown in Figure 3. The beets were significantly higher in glutamic acid content under the low (M_0) moisture treatment than under the medium (M_1) and high (M_2) moisture treatments. However, no significant difference was found between the M_1 and M_2 treatments. Each increase in soil moisture depressed the glutamic acid content in both varieties at both harvest dates. For any given nitrogen regime an increase in available moisture tended to depress the glutamic acid content (Figure 2). This

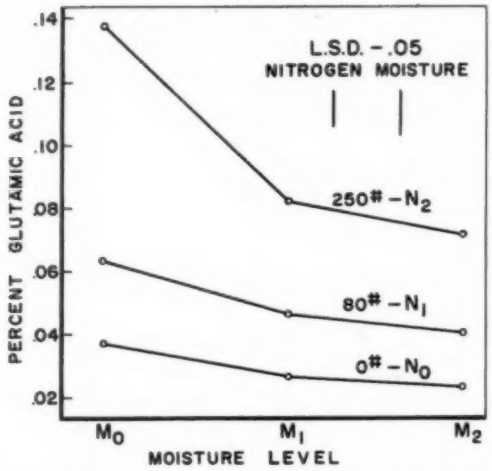

Figure 2.—Influence of soil moisture and nitrogen fertilization on the glutamic acid content of sugar beets, Logan, Utah, 1955 (Percentages given are averages over both varieties and both harvest dates).

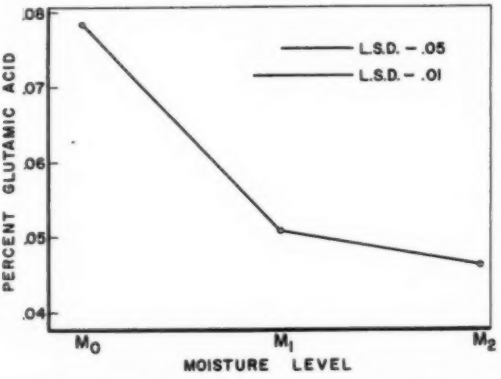

Figure 3.—Influence of soil moisture on the glutamic acid content of the sugar beet, Logan, Utah, 1955. (Percentages given are averages over both varieties and both harvest dates).

was most apparent at the high (N_2) fertility level and at the lower moisture levels. These results are in agreement with the work of Walker and Hac (8) who in cooperation with Haddock found that under any given nitrogen regime, an increase in available moisture tended to depress the glutamic acid content.

Haddock (4) reported a corresponding effect on many of the other nitrogenous components of the sugar beets.

Any difference in glutamic acid content due to moisture treatment must be attributed to the total amount of water applied and to the number of irrigations given each plot. The difference in the total amount of water applied in the three (M_0, M_1, and M_2) moisture treatments is indicated in Table 1. The highly significant difference between the M_0 and M_1 treatments can probably be attributed to the three additional inches of water and to the two additional irrigations that the M_1 plots received. If this is true, then it can be concluded that a delicate relationship exists between glutamic acid and soil moisture. These data would suggest that the M_2 moisture level was near the optimum for glutamic acid suppression. They also indicate that further studies using higher soil moisture tensions (approaching the wilting percentage) would be advisable. Walker and Hac (8) and Haddock (4) found that the glutamic acid content of sugar beets increased with increasing moisture tensions up to about eight atmospheres.

The differences due to harvest date and variety were non-significant. However, there was a slight consistent reduction in glutamic acid at the late harvest date. Variety US 22 Improved was slightly higher in glutamic acid than Stewart's 53104-0.

Summary

A factorial field experiment was conducted at Logan, Utah, in 1955 to study the influence of soil moisture, nitrogen fertilization, variety, and harvest date on the glutamic acid content of sugar beets. Highly significant differences were found between the low (M_0) and the medium (M_1) as well as between the low (M_0) and the high (M_2) moisture treatments. The glutamic acid content was depressed with each increase in soil moisture.

Highly significant differences in the glutamic acid content of sugar beets were found between low (0 pounds N) medium (80 pounds N) and high (250 pounds N) nitrogen levels. The glutamic acid content increased in a linear manner with each increment of nitrogen fertilizer.

The effects of varieties and harvest dates on glutamic acid content were small and non-significant under the conditions of this experiment.

The nature of the relation between nitrogen fertilization and glutamic acid response suggests that this may be a useful method of determining the nitrogen needs of the sugar beet.

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Population Genetic Studies on the Total Nitrogen in Sugar Beets (Beta Vulgaris L.)[1, 4]

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The increased use of nitrogen fertilizers in the production of sugar beets has emphasized the importance of chemical genetic studies involving the amount of nitrogen in the "thin juice" obtained during the processing of the sugar beet in the manufacture of sugar. Population genetic studies were conducted on the milligrams of nitrogen per 100 milliliters of thin juice equated to a refractive dry substance of 10. The analyses were made on the thin juice derived from the root of the sugar beet (*Beta vulgaris* L.).

Material, Experimental Design, Methods, and Analyses

Materials

The populations are A54-1, A54-1BB, 50-406, 50-406BB, F_1 hybrid, and 52-307. A54-1 is a commercial variety and A54-1BB resulted from seed harvested from 25 mother beets of A54-1. The 25 mother beets giving rise to population A54-1BB were grown in an isolated seed plot along with 25 mother beets for each of 22 other populations. This isolation plot was composed of 25 rows. One mother beet from each population occurred at random in each row, making a total of 23 mother beets per row. Seed was harvested from all mother beets on an individual plant basis. Seed from each mother beet of A54-1 was bulked and thoroughly mixed to produce the seed lot from which A54-1BB was grown. Seed saved from mother beets of 50-406 and handled in a similar manner produced the population designated as 50-406BB. Populations 50-406 and 52-307 are inbreds and the F_1 hybrid population resulted from crossing them. The inbred 50-406 has green hypocotyls and 52-307 has red hypocotyls. The seed used to produce the F_1 hybrid plants was harvested from mother beets of 50-406, and the plots of the F_1 hybrid population were thinned to red-hypocotyl plants.

There were two treatments, fertilized and non-fertilized. The fertilized plots received a surface application of 100 pounds of

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[3] Geneticist, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture.

[4] The writers are indebted to R. Ralph Wood of The Great Western Sugar Company for obtaining thin juice samples, by an oxalate method standard with his company and also acknowledge the cooperation of D. W. Robertson, Robert S. Whitney, and W. R. Schmehl in conducting the field experiment.

N (nitrogen) and 250 pounds of P_2O_5 (phosphorus pentoxide) per acre on April 4, 1956. The fertilizer was cultivated under with a rototiller. The plots were planted on April 10 and 11. On June 26, another 100 pounds of N per acre was drilled in the center of each space between rows of the fertilized plots.

Experimental Design

The sources of variation and number of items are shown in Table 1. There are 40 replications. Treatments are a split plot of replications; the two treatments are randomized within each replication. The six populations are randomized within each treatment and the eight locations are within each plot of each population. Each location within a plot is composed of a single plant. The plots were single rows running from north to south. Hence, any differences due to locations represent a gradient running from north to south. Locations are a split plot of populations and the design is a split-split plot.

Table 1.—Sources of Variation Designated as Main Effects, and Number of Each.

Main Effects	Number
Replications	40
Populations	6
Treatments	2
Locations	8

Every plot was bordered by a row of A54-1. After thinning, each plot had 12 plants and the stand remained excellent during the growing season. Data were taken on only eight plants, the extra plants at each end of the plot being discarded at time of harvest. The rows were spaced 22 inches apart and the plants were thinned to a spacing of 20 inches within the row. Selection was avoided during thinning by saving the plant farthest south in a "blocked group."

Methods

The total nitrogen was determined on thin juice from individual beet roots. The method used was a modified micro-Kjeldahl nesslerization (2)[5]

Micro-Kjeldahl digestion

Frozen juice was thawed and thoroughly mixed. To a pyrex glass digestion tube, marked at 35 and 50 ml, were added 0.5 ml of thin juice, 1.0 ml of oxidizing solution[6] and four glass beads.

[5] Numbers in parentheses refer to literature cited.

[6] Oxidizing agent. Dissolve 10 g of sodium molybdate in 150 ml of distilled water. Add slowly 150 ml of concentrated sulfuric acid. Cool, and add 200 ml of perchloric acid (70-72%). Then mix well. For details of this method see Bolin, King, and Klosterman (1).

This may stand over night if a large number of samples are being run. It is digested from 20-30 minutes on medium or low heat.[7, 8]

Nesslerization

The nesslerization process followed the modified techniques of G. W. Plaut (Enzyme Institute, University of Wisconsin, unpublished data). Nessler's reagent was prepared according to Koch and McMeekin (2).

The digestion tube was cooled and 15 ml of distilled water were added. To this tube were added 1 ml of 2% gum ghatti and 8 ml of Nessler's solution. The Nessler's solution was blown directly into the middle of the tube. The mixture was made up to 50 ml with distilled water and rotated for mixing.

Maximum color is reached after about 10 minutes. The optical density of the solution was read at a wave length of 490 millimicrons in a Beckman Model B spectrophotometer. Standard solutions were prepared using ammonium sulfate in the concentration range of 10-500 micrograms per ml. The micrograms of nitrogen per ml were plotted as the ordinate and the optical density as the abscissa. The slope of the straight line was used as a factor with the optical density reading of the sample to determine total nitrogen. The total nitrogen was reported as milligrams of nitrogen per 100 ml of thin juice with an RDS (refractive dry substance) of 10. For example:

$$\frac{10 \text{ RDS}}{13.14 \text{ (RDS of sample)}} \times 42.00 \text{ (mg of nitrogen per 100 ml of sample)} = 32.00 \text{ mg of nitrogen per 100 ml of thin juice with an RDS of 10.}$$

Analyses

The methods used in analyzing the data are those given by Powers (3, 4, 5) and Powers et al. (6, 7, 8, 9); for details of the calculations, the reader is referred to these articles. Primarily the analyses were made by employing the analysis of variance, the partitioning and components of variance methods of genetic analysis, and regression.

Results

The results are discussed under the general headings: means, variances, and frequency distributions. The means and variances are divided further into main effects and interactions. Comparisons made in the discussion of the data involve significant differ-

[7] No explosions have resulted with this type of heat.

[8] A study was made of the possible error introduced by the presence of inorganic nitrogen in the samples. It was concluded that the digestion process picks up some inorganic nitrogen in samples of low total nitrogen but in samples of high total nitrogen the inorganic nitrogen had little effect.

ences unless otherwise stated. For sake of clarity in emphasizing the biological deductions, tests of significance will not be given. In this article milligrams of nitrogen per 100 milliliters of thin juice equated to a refractive dry substance of 10 will be termed total nitrogen.

Table 2.—Analysis of Variance for Total Nitrogen, Data Converted to Logarithms.

Source of Variation	Smaller M. S.	Mean Square	Degrees of Freedom	F Value	F Value for 5% Level	F Value for 1% Level
Replications		1.5937b	39	8.27	1.69	2.11
Populations		9.0939a	5	121.90	2.26	3.11
Treatments		129.2414b	1	670.69	4.08	7.31
Locations		0.0338c	7	1.38	2.05	2.73
R X P	a	0.0746d	195	1.06	1.26	1.39
R X T	b	0.1927d	39	2.74	1.45	1.69
P X T	c	0.9981d	5	14.18	2.26	3.11
R X P X T	d	0.0704e	195	2.87	1.17	1.25
Remainder	e	0.0245	3288[1]			

[1] Degrees of freedom for remainder reduced by 65, the number of plants for which there were no data; values replaced by average of corresponding plot

Means

Analysis of variance

The analysis of variance for total nitrogen is given in Table 2. The smaller mean squares used to calculate the F values are designated by the letters in column 2 and the letters following the mean square values in column 3. The data of individual plants were converted to logarithms. However, since the biologist is primarily interested in the data expressed on the arithmetic rather than the logarithmic scale, the arithmetic means are given. For these data the interpretation and biological conclusions are the same whether the data are expressed on the logarithmic or arithmetic scale. These facts are pointed out because those more interested in statistical procedure may prefer to use the logarithmic scale throughout to interpret the data and draw conclusions. The data were converted to logarithms because the data on the logarithmic scale followed the normal probability integral but did not do so on the arithmetic scale.

Whether there are significant differences between main effects and whether interactions are statistically significant can be determined by comparing the F values in column 5 with those in columns 6 and 7. There are significant differences for replica-

tions, populations, and treatments. The first order interactions of replications X treatments, and populations X treatments are significant. The second order interaction of replications X populations X treatments is significant.

Table 3.—Means for Total Nitrogen for Populations, Treatments, and Populations X Treatments.

Population	Treatment		Average
	Fertilized	Non-Fertilized	
	mg	mg	mg
A54-1	46.8	18.8	32.8
A54-1BB	44.8	16.9	30.8
Average	45.8	17.8	31.8
50-406BB	33.6	12.6	23.1
50-406	31.2	14.6	22.9
Average	32.4*	13.6	23.0
F_1 hybrid	21.3	9.8	15.6
52-307	18.6	11.1	14.8
Average	20.0	10.4	15.2
Grand average	32.7	14.0	23.3

Main effects

The means of the main effects are listed in Table 3. The F values for populations and treatments compared with their respective F values at the 1 percent and 5 percent levels of significance reveal that there are significant differences between populations and between the two treatments. There are three levels of total nitrogen for populations. Populations A54-1 and A54-1BB compose the high level, 50-406 and 50-406BB the intermediate level, and the F_1 hybrid and 52-307 the low level. The data for 50-406, the F_1 hybrid, and 52-307 furnish evidence as to dominance relations in this cross. The average values for these three populations are 22.9, 15.6, and 14.8, respectively. Clearly in the cross produced by hybridizing the two inbreds (50-406 and 52-307), on an average, lower total nitrogen in the thin juice is nearly if not completely dominant. This deduction is supported to some extent by the values of 23.1 and 22.9 for populations 50-406BB and 50-406 and perhaps to a lesser extent by the values of 32.8 and 30.8 for populations A54-1 and A54-1BB.

If the data from A54-1 and A54-1BB support the complete dominance hypothesis, then on an average the varieties, strains, and inbreds which acted as male parents to produce the A54-1BB population averaged higher in total nitrogen than did A54-1.

First- and second-order interactions

The first order interaction of populations X treatments is shown in Table 3. The differences between populations belonging to different levels of total nitrogen are greater on the fertilized plots than differences involving corresponding camparisons on the non-fertilized plots. For example (45.8-20.0)-(17.8-10.4) giving a value of 18.4 is significantly different from zero. In testing significance t was employed and 18.4 is divided by 2 and the appropriate standard error. Similar comparisons within levels do not approach statistical significance and the differences involved are of such small magnitude, comparatively, as to have little if any practical significance even though the differences were statistically significant. Of considerable practical importance to a breeding program is the fact that the amount of total nitrogen for population A54-1 on the non-fertilized plots is not materially different from the amount of total nitrogen for the F_1 hybrid and 52-307 populations on the fertilized plots. That is, the values of 18.8, 21.3, and 18.6 are not significantly different. Hence, the differences due to treatments and differences due to populations are approximately of the same magnitude.

Such a finding lends considerable encouragement to the plant breeder as it provides evidence that beets can be bred for either low or high nitrogen content. Further it shows that in this study genetic control (represented by populations) of total nitrogen is of about the same magnitude as the environmental control (represented by the two fertilizer treatments).

The replications X populations X treatments means for total nitrogen are listed in Table 4. For clarity in presentation, the data for 8 contiguous replications are grouped and presented as averages. This makes five groups for the 40 replications. The differences in total nitrogen between populations are greatest on the fertilized plots and for the replication group 33-40. In other words the differences between populations are greatest for the replication group and fertilizer treatment having the highest concentration of total nitrogen in the thin juice. Another observation of interest is that on both the fertilized and non-fertilized plots the F_1 hybrid has more nitrogen in the thin juice for those replication groups averaging more than 23 mg of total nitrogen (per 100 ml of thin juice) than does the inbred parent 52-307. The reverse is true for those replication groups averaging less than 23 mg of total nitrogen (see non-fertilized, Table 4). That is, for the replication groups 1-8, 9-16, 17-24, and 25-32 on the non-fertilized plots the F_1 hybrid has less total nitrogen

Table 4.—Means of Total Nitrogen per 100 ml for the Interaction of Replications X Populations X Treatments.

Treatment and Population	Replication Groups					Average
	1-8	9-16	17-24	25-32	33-40	
Fertilized						
	mg	mg	mg	mg	mg	mg
A54-1	38.59	34.99	38.85	45.90	75.79	46.81
A54-1BB	34.81	37.42	33.87	50.80	67.05	44.79
50-406BB	26.21	25.71	28.01	27.64	60.35	33.58
50-406	26.35	24.25	28.28	30.53	46.80	31.24
F_1 hybrid	17.41	17.86	18.83	20.32	32.23	21.33
52-307	14.05	16.12	17.36	19.90	25.71	18.63
Average	26.23	26.06	27.53	32.52	51.32	32.73
Non-fertilized						
A54-1	13.78	12.54	11.89	19.77	36.27	18.85
A54-1BB	12.54	13.15	12.50	17.46	29.04	16.94
50-406BB	8.92	10.87	9.31	11.08	22.92	12.62
50-406	10.96	11.29	10.52	14.66	25.39	14.56
F_1 hybrid	8.49	8.73	7.27	9.84	14.75	9.82
52-307	9.02	10.79	8.43	12.55	14.55	11.07
Average	10.62	11.23	9.99	14.23	23.82	13.98
Grand average	18.43	18.65	18.76	23.38	37.57	23.36

than either inbred parent, 50-406 or 52-307. Thus heterosis for less nitrogen is occurring at the lower fertility levels.

The greatest difference between populations within a fertilizer treatment (75.79-25.71), or 50.08, is greater than the greatest difference between fertilizer treatments within a population (75.79-36.27), or 39.52. The genetic difference represented by populations is larger than the environmental difference represented by treatments. Hence the means for the interaction of replications X populations X treatments support the deduction from the interaction of populations X treatments. These results provide convincing evidence that beets can be bred under the conditions of this experiment for either low or high nitrogen content in the thin juice. This is particularly true for the fertilized plots and for replications in which larger quantities of nitrogen are available to the sugar beet plant.

Variances

Analysis of variance of the within-plot variances

The analysis of variance of the within-plot variances for total nitrogen in the thin juice is given in Table 5. The original data were converted to logarithms. Whether there are significant

Table 5.—Analysis of Variance of the Variances for Total Nitrogen; Data Converted to Logarithms.

Source of Variation	Mean Square	Degrees of Freedom	F Value	F Value for 5% Level	F Value for 1% Level
Replications	0.00046638	39	1.41	1.45	1.69
Populations	0.00632655	5	19.10	2.26	3.11
Treatments	0.00040307	1	1.22	3.89	6.76
R X P	0.00039174	195	1.18	1.26	1.39
R X T	0.00036187	39	1.09	1.45	1.69
P X T	0.00063976	5	1.93	2.26	3.11
R X P X T	0.00033115	195			

differences for main effects can be determined by comparing the F values in column 4 with those in columns 5 and 6. An examination of Table 5 reveals that the only significant differences are between populations and that these differences are highly significant. The large F value for populations could be due to differences between segregating and non-segregating populations. It is desirable to know whether the differences between populations in the homogeneous material are statistically significant. An analysis of variance of the variances involving only the data for homogeneous populations was made. The procedures used in making the analysis are the same as that shown in Table 5 with the exception that the number of populations is 3 instead of 6. The F value for populations in this latter test is 5.88 and that for the 1% level is 4.88. At least some of the differences between the non-segregating populations are statistically significant. This information is useful in deciding whether to use regression in estimating the environmental variances of the heterogeneous populations.

The means and within-plot obtained, estimated, and residual variances [(R X L) + L] for total nitrogen are listed in Table 6. That conversion of the original data to logarithms normalized it will be shown later during the analyses of the frequency distributions. Methods of testing the reliability of the residual variances are provided by Powers, Robertson, and Remmenga (8).

Regression was used to estimate the environmental variances of Table 6. Regression was calculated within fertilizer treatments and hence n is 3 instead of 6 as was the case in the article by Powers et al (8). This information is necessary if the reader wishes to calculate the values in column 4 of Table 6.

Table 6.—Means and Within-plot Obtained, Estimated, and Residual Variances [(R X L) + L] for Total Nitrogen for Treatments and Populations; Data Converted to Logarithms.

Treatments and Populations	Means	Variance		
		Obtained	Estimated	Residual
Fertilized				
A54-1	1.607108	0.037264	0.024402	0.012862
A54-1BB	1.587479	0.037772	0.023768	0.014004
50-406BB	1.445778	0.031687	0.019188	0.012499
50-406	1.453922	0.019888	0.019451	0.000437
F_1 hybrid	1.291699	0.012584	0.014208	—0.001624
52-307	1.231595	0.013452	0.012265	0.001187
Non-fertilized				
A54-1	1.183687	0.027436	0.023115	0.004321
A54-1BB	1.156908	0.032528	0.022338	0.010190
50-406BB	1.023635	0.026817	0.018473	0.008344
50-406	1.097637	0.020946	0.020619	0.000327
F_1 hybrid	0.947119	0.016740	0.016255	0.000485
52-307	1.007105	0.017182	0.017994	—0.000812

An examination of the variances and means for populations 50-406, F_1 hybrid, and 52-307 (homogeneous populations) reveals that on the whole there is a positive relation between the means and obtained variances within treatments. For these populations this relation does not hold between treatments. That is, the means are larger on the fertilized plots than on the non-fertilized plots and the reverse is true for the variances. Consequently the within-treatment regressions were used to estimate the environmental variances.

Linear regression of the obtained variances on the means for the non-segregating populations (50-406, F_1 hybrid, 52-307) accounted for 87.5 percent of the variability of the variances on the fertilized plots and 90.9 percent of that on the non-fertilized plots. The use of linear regression to estimate the environmental variances of the segregating generations is justified.

The residual variances listed in column 5 of Table 6 are significantly different from zero for the segregating populations (A54-1, A54-1BB, and 50-406BB), indicating considerable genetic variability in these populations for total nitrogen in thin juice. The residual variances for population A54-1BB are larger than those for A54-1 on both the fertilized and non-fertilized plots. Apparently exposing mother beets of A54-1 to pollination by 22 other varieties or lines has resulted in a broader genetic base.

This finding has a bearing on the interpretation of the data as it indicates that a study of the frequency distributions will yield information of biological importance.

Frequency Distributions

The obtained frequency distributions of populations for total nitrogen in the thin juice expressed in numbers are listed in Table 7. The total number of individuals for each population within a fertilizer treatment is 320. In analyzing the data it is desirable to know whether, on the arithmetic scale, the environmental variability is following the normal probability integral. Goodness-of-fit chi squares were calculated for the non-segregating populations using the arithmetic scale. All the P values fell between 0.01 and 1—infinity. It is evident that the environmental variability is not following the normal probability integral on the arithmetic scale. An examination of Table 7 reveals that for both the segregating and non-segregating populations the data are skewed toward the lower values of the frequency distributions. This suggests that the data may be following the logarithmic scales.

Chi squares for goodness of fit were calculated to determine whether the environmental and genetic variabilities are following the logarithmic scale. For details of the methods employed here and immediately above see Powers (5). The results of the tests to determine whether the environmental variability and both the environmental and genetic variabilities are following the logarithmic scale are given in Table 8.

An examination of Table 8 reveals that for the non-segregating populations none of the P values are as low as 0.05 and that the total values for the two fertilizer treatments and grand total have P values falling between 0.20 and 0.10. From these results it seems logical to conclude that the data measuring the environmental variability follow the normal probability integral after conversion to logarithms. Such is not the case for the segregating populations. The only population having P values larger than 0.05 on both the fertilized and non-fertilized plots is A54-1BB. This indicates that the genetic variability for populations A54-1 and 50-406BB are not following the normal probability integral, whereas both the environmental and genetic variabilities are doing so for population A54-1BB. This difference in behavior between populations could be due to the segregation of comparatively few genes conditioning total nitrogen in populations A54-1 and 50-406BB, whereas more genes conditioning total nitrogen are segregating in population A54-1BB.

Table 7.—Frequency Distributions of Treatments and Populations for Total Nitrogen, Expressed in Numbers.

Treatment and Population	Upper Limit of Class, mg of N per 100 ml of Thin Juice																		
	0 to 7	14	21	28	35	42	49	56	63	70	77	84	91	98	105	112	119	126	133	140 and Over
	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No	No.	No.	No.	No.	No.	No.	No.
Fertilized																				
A54-1		9	31	44	48	34	32	31	18	18	7	14	8	6	11	4	3		1	1
A54-1BB		12	29	42	47	48	37	32	12	14	13	7	8	5	6		2	3	1	2
50-406BB	1	31	80	66	42	25	19	9	12	8	8	1	6	2	5		2	1	1	1
50-406		11	72	71	75	30	28	13	6	5	4	2	2				1			
F_1 hybrid	1	64	135	73	22	10	3	6	3	2	1									
52-307	5	94	125	57	22	12	3	2												
Non-fertilized																				
A54-1	21	138	90	31	9	7	4	5	5	1	2	1	2	2		2				
A54-1BB	22	141	92	35	16	2	2	2		2	3			2		1				
50-406BB	55	199	40	7	8	4		1		2	1	1	1	1						
50-406	33	169	76	22	9	1	3	1	3		2		1							
F_1 hybrid	92	189	23	8	6	1	1													
52-307	55	202	47	14	1	1														

Table 8.—Goodness-of-Fit Chi Squares and P Values to Test Normal Distribution of the Frequencies for Total Nitrogen, Data Converted to Logarithms, Segregating and Non-Segregating Populations.

Segregation Status, Treatment, and Population	Chi Square	Degrees of Freedom	P Lies Between
Segregating			
Fertilized			
A54-1	17.5818	11	0.10 and 0.05
A54-1BB	9.7961	11	0.70 and 0.50
50-406BB	20.0924	9	0.02 and 0.01
Total	47.4703	31	0.05 and 0.02
Non-fertilized			
A54-1	31.6652	6	0.01 and 1—infinity
A54-1BB	5.9551	5	0.50 and 0.30
50-406BB	38.9728	4	0.01 and 1—infinity
Total	76.5931	15	0.01 and 1—infinity
Grand total	124.0634	41	0.01 and 1—infinity
Non-segregating			
Fertilized			
50-406	10.4791	7	0.20 and 0.10
F_1 hybrid	8.0646	4	0.10 and 0.05
52-307	1.4626	4	0.90 and 0.80
Total	20.0063	15	0.20 and 0.10
Non-fertilized			
50-406	8.2790	4	0.10 and 0.05
F_1 hybrid	1.4296	2	0.50 and 0.30
52-307	3.1227	3	0.50 and 0.30
Total	12.8313	9	0.20 and 0.10
Grand total	32.8376	24	0.20 an 0ad.10

The obtained and calculated frequency distributions, differences, and proportions of genetic deviates expected are listed in Table 9. For the method of analyzing the data shown in Table 9, see Powers, Robertson, and Clark (7) and Powers et al (9). The totals for number of genetic deviates expected in the lower classes of Table 9 are listed in the second to last column and those in the higher classes of Table 9 in the last column. The standard error of the binomial distribution may be used to determine whether the populations differ as to number of genetic deviates in the lower classes and the higher classes. Also

Table 9.—Obtained and Calculated Frequency Distributions and Differences and Proportions of Individuals in the Lower Classes and in the Higher

Table 9.—Obtained and Calculated Frequency Distributions and Differences and Proportions of Individuals in the Lower Classes and in the Higher Classes Expected to be Genetic Deviates for Total Nitrogen; Data Converted to Logarithms, Fertilized and Non-Fertilized Plots, and Segregating Populations.

Treatment, Population, and Distribution	Upper Limit of Class (mg per 100 ml) and Logarithms																				Genetic Deviates	
	(0 to 7)	(14)	(21)	(28)	(35)	(42)	(49)	(56)	(63)	(70)	(77)	(84)	(91)	(98)	(105)	(112)	(119)	(126)	(133)	(140)	Lower Values	Higher Values
	0 to 0.8451	1.1461	1.3222	1.4472	1.5441	1.6232	1.6902	1.7482	1.7993	1.8451	1.8865	1.9243	1.9590	1.9912	2.0212	2.0492	2.0755	2.1004	2.1239	2.1461		
Fertilized	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.
A54-1																						
Obtained		9	31	44	48	34	32	31	18	18	7	14	8	6	11	4	3		1	1	84	73
Calculated		1	11	38	61	62	52	36	24	14	9	5	3	2	1	1	0		0	0	50	35
Difference		8	20	6						4	—2	9	5	4	10	3	3		1	1	34	38
Proportion		0.89	0.65	0.14						0.22		0.64	0.62	0.67	0.91	0.75	1.00		1.00	1.00	0.40	0.52
A54-1BB																						
Obtained		12	29	42	47	48	37	32	12	14	13	7	8	5	6		2	3	1	2	41	61
Calculated		1	13	44	67	64	50	33	21	12	7	4	2	1	1		0	0	0	0	14	27
Difference		11	16							2	6	3	6	4	5		2	3	1	2	27	34
Proportion		0.92	0.55							0.14	0.46	0.43	0.75	0.80	0.83		1.00	1.00	1.00	1.00	0.66	0.56
50-406BB																						
Obtained	1	31	80	66	42	25	19	9	12	8	8	1	6	2	5		2	1	1	1	112	56
Calculated	0	5	55	101	82	44	20	8	3	1	1	0	0	0	0		0	0	0	0	60	13
Difference	1	26	25					1	9	7	7	1	6	2	5		2	1	1	1	52	43
Proportion	1.00	0.84	0.31					0.11	0.75	0.88	0.88	1.00	1.00	1.00	1.00		1.00	1.00	1.00	1.00	0.46	0.77
Non-fertilized																						
A-54-1																						
Obtained	21	138	90	31	9	7	4	5	5	1	2	1	2	2		2					159	31
Calculated	4	124	134	45	10	2	1	0	0	0	0	0	0	0		0					128	3
Difference	17	14				5	3	5	5	1	2	1	2	2		2					31	28
Proportion	0.81	0.10				0.71	0.75	1.00	1.00	1.00	1.00	1.00	1.00	1.00		1.00					0.19	0.90
A54-1BB																						
Obtained	22	141	92	35	16	2	2	2		2	3			2		1					22	30
Calculated	6	145	126	34	7	2	0	0		0	0			0		0					6	9
Difference	16				9	0	2	2		2	3			2		1					16	21
Proportion	0.73				0.56	0	1.00	1.00		1.00	1.00			1.00		1.00					0.73	0.70
50-406BB																						
Obtained	55	199	40	7	8	4		1		2	1	1	1	1							55	26
Calculated	30	231	55	4	0	0		0		0	0	0	0	0							30	4
Difference	25			3	8	4		1		2	1	1	1	1							25	22
Proportion	0.45			0.43	1.00	1.00		1.00		1.00	1.00	1.00	1.00	1.00							0.45	0.85

homogeneity chi square can be employed for this purpose (Powers 3, 4) and perhaps is both easier to apply and more comprehensive.

There are no significant differences between populations with regard to the number of genetic deviates in the higher classes (last column of Table 9). However, the numbers of genetic deviates in the higher classes for both treatments and all populations are significantly different from zero.

Turning to a consideration of differences between populations for the lower classes (second to the last column of Table 9) there are significant differences between fertilizer treatments and between populations for both fertilizer treatments. The non-fertilized plots are lower in number of genetic deviates. These data furnish some evidence that breeding for low nitrogen at the higher fertility level would be more effective. A54-1BB is lower in genetic deviates than either of the other two populations. Again all of the numbers of genetic deviates are significantly different from zero for all populations in both treatments.

From these considerations breeding within these three populations for either low, intermediate, or high nitrogen would be expected to result in some genetic improvement and would be more effective at the higher fertility level.

The proportions of genetic deviates in the different classes should be considered in selecting individuals for further breeding. This is particularly true if selection is employed as the breeding method. Those classes having 100 percent genetic deviates would be of particular interest as they allow the identification (with reasonable limits) of the genetic deviates. A progeny test is required to identify the genetic deviates selected from those classes having less than 100 percent genetic deviates. Of course those individuals selected from classes showing 100 percent genetic deviates should be progeny tested also as occasionally chance environmental deviates would be expected to occur among them.

An examination of the frequency distribution of the genetic deviates for population A54-1 reveals that this frequency distribution has a mode among the lower deviates and another among the higher deviates. These modes have upper class limits of 21 and 105, respectively. Such being the case the mean total nitrogen for each of these two groups can be estimated. The procedure will be illustrated for the lower classes. The class centers may be obtained by dividing the class interval

7 by 2 (equals 3.5) and adding this value to the value for the immediately preceding class. By so doing the class centers are 10.5, 17.5, and 24.5. The frequencies for these classes taken from Table 9 opposite row heading differences are 8, 20, and 6, respectively. Multiplying the class centers by their respective frequencies and summing we have [(8 X 10.5) + (20 X 17.5) + (6 X 24.5)] and this divided by 34 equals 17, the mean for the lower classes. Similarly the mean for the higher classes is estimated as 94. It should be possible to obtain by breeding, a population having a mean of 17 and another population having a mean of 94. The latter population would have 5 times as much total nitrogen as the former. The mean of population A54-1 for the fertilized plots as shown in Table 3 is 46.8. The decrease in total nitrogen for the first population is 275 percent and the increase for the second population is 201 percent.

Summary

1. The unit of measurement is milligrams of nitrogen per 100 milliliters of thin juice equated to a refractive dry substance of 10.

2. Significant differences in amounts of total nitrogen were found between populations and between the two fertilizer treatments. Populations represent three levels of total nitrogen: A54-1 and A54-1BB, 31.8; 50-406BB and 50-406, 23.0; and F_1 hybrid and 52-307, 15.2.

3. Nearly complete dominance of smaller amounts of total nitrogen was found for those replication groups in which the total nitrogen averaged over 23 whereas heterosis for smaller amounts of nitrogen was found for those replication groups in which the total nitrogen averaged less than 23.

4. The amount of total nitrogen for population A54-1 (18.8) on the non-fertilized plots is not materially different from the amount of total nitrogen for 52-307 (18.6) and F_1 (21.3) on the fertilized plots. Hence in this study genetic control of total nitrogen represented by differences between populations is of about the same magnitude as environmental control represented by differences between fertilizer treatments.

5. The differences between populations in amount of total nitrogen are greatest for the replication group 33-40 which had the greatest amount of nitrate nitrogen in the petioles (see Powers et al. 9). This is substantiated also by the fertilizer treatments as the range for populations for the fertilized plots was 18.6 to 46.8 and for non-fertilized plots was 9.8 to 18.8.

6. Such findings lend considerable encouragement to the plant breeder, as they provide evidence that beets can be bred for either low or high nitrogen content. Furthermore, the accomplishments are expected to be more marked at the higher fertility level, for which improved varieties are much needed.

7. The residual variances for populations A54-1, A54-1BB, and 50-406BB were greatly different from zero, indicating that considerable genetic variability existed in these populations. The partitioning method of analyzing the frequency distributions confirmed this deduction and showed that breeding within these populations and between the six populations should be effective. It was found that breeding within A54-1 should result in the production of populations differing by five times in the amount of total nitrogen.

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Field Inoculation of Sugar Beets with Aphanomyces Cochlioides Drechs[1]

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Introduction

In field tests to evaluate the resistance of sugar beet strains to black root caused by the water mold, *Aphanomyces cochlioides* Drechs., a severe disease exposure is desirable. In tests made on land successively cropped to sugar beets, extreme local differences in degree of disease exposure are often evident. One apparent requirement for uniform disease exposure is uniform distribution of the pathogen in the plots. The present study was made, therefore, to develop methods of introducing artificial inoculum of *A. cochlioides* to field plots in order to insure a uniform disease exposure.

No reports were found in the literature concerning methods of inducing extreme field exposures to *A. cochlioides*. Jones and Drechsler (1)[3] obtained limited infection of peas with the root-rot fungus, *Aphanomyces euteiches* Drechs., when the following kinds of inoculum were applied in the field: greenhouse soil previously inoculated with cultures of the fungus, zoospores, and cultures rich in oospores. Kendrick (2) obtained infection of radishes when he spread soil infested with the root-rot fungus, *Aphanomyces raphani* Kendr., on a disease-free plot area before planting.

Materials and Methods

Preliminary studies were made to determine substrates suitable for growing field inoculum. Since *A. cochlioides* requires a relative humidity of at least 98 percent in its immediate environment for growth (3), it follows that the substrate should be capable of retaining sufficient moisture for growth of the fungus. The number of plots to be inoculated in field tests is sometimes too great to make hand application of inoculum feasible; hence, it is desirable that the substrate be of size and consistency that permit quick and uniform application with a mechanical device, such as a planter or fertilizer distributor.

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[3] Numbers in parentheses refer to literature cited.

The following substrates came nearest to fulfilling these requirements and were tested separately in the field after preliminary greenhouse tests:

1. Steamed soil, infested with cultures of the fungus.

2. Oat-grain medium, prepared by adding approximately 250 ml. each of oat grains and water to one-quart fruit jars and sterilizing for 2 hours on 2 successive days.

3. Sorghum-grain medium, prepared according to the method outlined by Whitehead (4).

In each test, the inoculum was applied in the drill row with a V-belt planter. The seed of all sugar beet varieties included in the tests was treated with a fungicide to reduce the incidence of pre-emergence damping-off caused by fungi other than *A. cochlioides.*

Experimental Results and Discussion

In a field test at St. Paul, Minnesota, in 1954, artificially infested soil was applied in the drill row at the rate of approximately one-fourth pint per 10 feet of row. Immediately after application of inoculum, a sugar beet variety susceptible to black root was planted with a Planet Jr. hand planter.

Reduced stands and occurrence of black root in inoculated plots indicate that infection resulted from the inoculation treatment (Table 1). The disease exposure was not so great as was desired, however, as it was less than that which occurred naturally in a nearby field planted with the same variety but where successive crops of sugar beets had been grown.

Table 1.—Artificial Inoculation of Field Plots of Sugar Beets with *Aphanomyces cochlioides:* Infested Soil Applied by Planter before Seeding.[1]

Treatment	Plants per Plot		Plants with Black Root at Thinning	Yield of Roots
	Before Thinning	At Harvest		
	Number	Number	Percent	Pounds
Artificially infested soil	45	19	25	31.4
Control (no treatment)	87	31	0	48.4
L.S.D. (P = 0.05)	25	6		9.8

[1] Data expressed as means of five 4-row plots, each 10 feet long.

In a field test at Waseca, Minn., in 1955, oat-grain inoculum was applied in the drill row in soil naturally infested with *A. cochlioides.* Seed of a black-root-resistant variety and of a sus-

ceptible variety were planted with the oat-grain inoculum. Controls consisting of 1) noninfested oat grains and 2) no treatment were included. The oat grains were placed on top of the seed on the V-belt and applied at the rate of 75 ml. per 20 feet of row. As the oat-grain inoculum tended to be wet and sticky, immediately before application it was mixed with dry sawdust which absorbed much of the excess moisture and was then removed with a screen.

Reduced stands indicate that incidence of black root increased upon application of the oat-grain inoculum at the time of planting (Table 2).

Table 2.—Artificial Inoculation of Field Plots of Sugar Beets with *Aphanomyces cochlioides:* Oat-Grain Inoculum Applied by Planter with Seed.[1]

Treatment	Inches Containing Plants in 240-Inch Row before Thinning		Plants per Plot at Harvest	
	US 400	S.P. 486-0	US 400	S.P. 486-0
	Number	Number	Number	Number
Oat-grain inoculum	79	59	13.6	9.8
Control (noninfested oat grains)	106	103	17.0	12.8
Control (no treatment)	113	109	14.6	13.6
L.S.D. (P = 0.05) for comparing:				
Varieties	16		2.0	
Treatments	22		2.8	

[1] Data expressed as means of 5 single-row plots, each 20 feet long.

In tests at the Plant Industry Station, Beltsville, Md., inoculation of sugar beet seedlings in cold frames was obtained upon mechanical application of 150 ml. of sorghum-grain inoculum per 20 feet of row. As in the 2 previously described tests, prevalence and severity of black root in infested plots was greater than in noninfested plots. The average stand of inoculated plots was reduced 58 percent, 73 percent of the surviving plants showing black-root symptoms in contrast to 23 percent of the surviving plants in noninoculated plots. In this test as in the others, seedlings with black-root symptoms when placed in Syracuse dishes containing a shallow layer of water, usually yielded *Aphanomyces cochlioides.*

Since each of the different inoculation media—infested soil, oat grains, and sorghum grains—was tested separately at different

times, in different places, and under different environmental conditions, no direct comparison can be made of their relative efficacy in field inoculation. The relatively lower degree of black root obtained with the infested soil inoculum may be attributed in part to the dry weather that prevailed during the early period of the test. In greenhouse experiments by McKeen (3), abundant black root occurred in sugar-beet seedlings grown in moist naturally infested soil (43% water content) but none occurred in dry soil (20% water content). Hence, it follows that adequate soil moisture is a requisite for successful field inoculation with *A. cochlioides*.

To insure uniform disease exposure, uniform distribution of the fungus in the inoculum substrate is essential. In the case of grain inoculum this can be ascertained visually; in the case of the soil inoculum it cannot. On the other hand, the infested soil inoculum is relatively easier to prepare and to maintain, especially in larger quantities.

The sorghum-grain inoculum, since it is less sticky than the oat-grain inoculum and has smaller kernels, is easier to apply through the planter.

Summary

Methods for field inoculation of sugar beet seedlings with *Aphanomyces cochlioides* are described. In field tests it was demonstrated that black-root incidence can be enhanced by mechanical application of artificial inoculum in the drill row at planting. This method is proposed as a means of increasing the uniformity of disease exposure in field tests.

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Partial Composition of Sugar Beet Molasses

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The need for more precise knowledge of the constituents of sugar beet molasses has been recognized for many years. The less we know about the composition of molasses and other processing liquors, the more we have to work blindly in developing logical methods for reducing molasses production or in finding further uses for the residues. Only after an accurate picture of molasses composition has been drawn will it be possible to adopt a rational evaluation of the melassigenic importance of various non-sugars. Molasses composition data may be applicable to crystallization studies, to genetic improvement of the beets, and in development of improved processing procedures. Feed formulators, fermenters, and others using molasses will find composition data valuable and new useful byproducts may be discovered.

Nine composite molasses samples have been analyzed for several of their constituents. Six of these samples were from straight house factories. The remaining samples were from factories using the Steffen process. The samples were obtained from Alvarado, Betteravia, Clarksburg, Manteca, and Woodland, California; Brighton, Colorado; Carrollton, Michigan; Moorhead, Minnesota, and Toppenish, Washington. The molasses samples were analyzed for potassium, total nitrogen, ammonia, amide and amino nitrogen, betaine, pyrrolidone carboxylic acid (PCA), lactic, glycolic, malic and citric acids, sulfur, phosphorus, chloride and total milliequivalents of anions. The results are presented on the basis of grams per 100 grams of non-sucrose solids.

Experimental

The molasses samples were diluted to 10 to 20 percent solids and the various determinations were made on aliquots of the diluted samples. Potassium was determined chemically utilizing the formation of insoluble potasium-sodium cobaltinitrite (1)[2]. Total and ammonia nitrogen were determined by conventional AOAC methods (2). It was later found on analyzing solutions containing known amounts of sucrose and potassium nitrate that the total nitrogen method used on these samples recovered

[1] Western Regional Research Laboratory, Western Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Albany California. Presented at a meeting of the American Society of Sugar Beet Technologists, Detroit, Michigan, Feb. 4-6, 1958.

[2] Numbers in parentheses refer to literature cited.

only 60 to 80 percent of the nitrate present. Based on this finding the total nitrogen determinations on the original molasses may be low by 4%. Acidic nitrogen determinations may be low by 8%. Amide nitrogen was determined by the method of Winton and Winton (3).

The method of Peters and Van Slyke was used to determine amino nitrogen (4). Betaine was determined by the method of Walker and Erlandsen (5). Pyrrolidone carboxylic acid (PCA) was calculated from a corrected nitrogen determination of the PCA fraction separated by ion exchange fractionation from other acidic nitrogen compounds (6). Citrate was determined by the pentabrom-acetone method (7). Lactic and malic acids were determined colorimetrically (8, 9). Glycolic acid was measured by an adaptation of the malic and lactic acid methods (10). Total halogen was determined by AOAC methods (2). Potter's method was used for estimation of total sulfur (11). Allen's method was used for estimation of total phosphorus (12).

The values for sulfur, chlorine, and phosphorus are presented as if they existed in molasses as sulfate, chloride, and phosphate. This assumption will introduce an error in the case of sulfur, since some of the sulfur will occur as sulfite or as a sulfur compound that will release sulfur dioxide on acidification and prolonged heating (13). Milliequivalents of anions were determined on an aliquot of dilute molasses by titration to pH 8.3 with sodium hydroxide solution after the cations were exchanged with hydrogen, using the cation exchange resin Dowex-50 (H).[3] Some acidic compounds such as glutamic and aspartic acids are also removed by the ion exchange resin. A nearly complete analysis of the amino acids from one sample of molasses (14) shows that the distribution of various amino acids in sugar beet molasses is quite different from the average distribution in protein. On the basis of the amino acid composition of sugar beet molasses a factor of 8.4 was calculated to convert amino nitrogen into amino acid content. Total amide is calculated as asparagine, since this amide is most likely to survive alkaline processing treatment, whereas the more common amide in sugar beets, glutamine, readily decomposes to form pyrrolidone carboxylic acid (PCA). Much of this ammonia is lost during the evaporation step in the manufacture of sugar.

Results

Results of the various analyses and calculations are presented in Tables 1 through 4. Table 1 presents a picture of the basic

[3] Mention of a specific product does not constitute endorsement by the Department of Agriculture over others of a similar nature not mentioned.

impurities or non-sucrose solids present in molasses. Table 2 shows the amounts of organic acids in molasses, while Table 3 gives the same data for inorganic acids and also the total milliequivalents of acids. Table 4 shows the average milliequivalents for each acid present in molasses and also the amount due to known organic and inorganic acids.

On the basis of data in Tables 1, 2, 3, and 4, the only apparent differences between the Steffen and straight house molasses are in the lower lactate content and, possibly, a lower potassium content of the Steffen molasses. The large variations in chloride content are of considerable interest, since Wise and Nicholson

Table 1.—Beet Molasses—Basic Constituents (g./100 g. non-sucrose solids).

Factory[1]	Potassium	Betaine	Amino acids[3]	Amide[4]
Al[2]	14.1	13.0	10	1.9
Be	14.6	16.9	11	2.8
Br	18.7	14.8	9	1.5
CaM	21.1	19.5	9	1.7
Cl	12.6	19.0	9	2.0
Ma	20.1	19.2	12	2.5
Mo	15.2	19.0	8	2.0
To[2]	13.5	20.4	11	2.5
Wo[2]	11.5	13.8	7	2.0
Average	15.7	17.3	10	2.1

[1] Abbreviations: Al = Alvarado, Be = Betteravia, Br = Brighton, CaM = Carrollton, Cl = Clarksburg, Ma = Manteca, Mo = Moorhead, To = Toppenish, and Wo = Woodland.

[2] Steffen molasses.

[3] Amino nitrogen times 8.4.

[4] Amide nitrogen calculated as asparagine.

Table 2.—Beet Molasses—Acidic Organic Constituents (g./100 g. non-sucrose solids).

Factory	Lactic	Glycolic	PCA	Citric	Malic
Al	3.3	2.1	6.1	1.5	1.8
Be	5.3	1.3	10.8	.0	1.2
Br	5.3	.8	8.3	2.5	2.6
CaM	5.4	1.7	6.7	.5	1.7
Cl	5.2	1.2	9.4	.5	1.4
Ma	6.6	1.7	13.4	.5	2.1
Mo	5.9	.8	9.0	1.5	2.1
To	2.3	1.3	14.5	1.3	1.9
Wo	3.1	1.9	6.4	1.0	2.2
Average	4.7	1.4	9.4	1.0	1.9

Table 3.—Beet Molasses—Acidic—Constituents.

Factory	Inorganic Anions (g./100 g. Non-Sucrose Solids)			Total Anions[1] (meq./100 g. Non-Sucrose Solids)
	Chloride	Sulfate	Phosphate	
Al	5.4	2.5	0.2	610
Be	6.7	4.0	0.2	560
Br	8.9	8.0	0.3	750
CaM	5.6	6.0	0.2	620
Cl	5.7	5.1	0.2	560
Ma	8.7	3.7	0.3	770
Mo	2.6	4.6	0.4	500
To	1.5	4.9	0.5	470
Wo	5.2	2.8	0.3	520
Average	5.6	4.6	0.3	600

[1] Includes organic and inorganic anions.

Table 4.—Average Milliequivalents of Anions (meq./100 g. non-sucrose solids)

Anions	Milliequivalents	
Lactate	52	
Glycolate	18	
PCA	73	
Citrate	16	
Malate	28	
Total Organic		187
Chloride	158	
Sulfate	96	
Phosphate	10	
Total Inorganic		264
Undetermined Acids		149
Total		600

(15), Moritsugu (16), and Rorabaugh and Norman (17) have pointed out that chloride as the potassium or sodium salt in a sucrose-water--chloride system decreases the rate of sucrose crystallization and increases sucrose solubility. These results have been confirmed in our laboratory by the work of Teranishi, Knowles, and McCready (18). They have shown by experiments with sucrose solutions containing a single impurity, that potassium sulfate had no measurable effect on crystallization rate but lowered the final solubility of sucrose in the mixture. On the other hand either potassium or sodium chloride lowered the rate of crystallization and increased sucrose solubility rate. The principal rate and solubility effects are apparently due to chloride.

The basic constituents form 45 percent of the total determined impurities. The principal unknown substances in this category are unidentified nitrogen compounds. Twenty-nine percent of the determined constituents are acids consisting of 18 percent organic acids and 11 percent inorganic acids. With the exception of nitrate most of the unknown or undetermined acids are probably organic. There are a few percent of raffinose, galactinol, and other essentially neutral compounds present. The known impurities that we have measured quantitatively average 74 percent of the non-sucrose solids and range from 58 to 89 percent for the samples investigated. The amounts of several known constituents of beet molasses such as sodium, raffinose, and nitrate were not measured in this study but a more complete analysis of representative molasses from the 1956 campaign is now under way and the average values for sodium, nitrate, and raffinose in straight house molasses are 3.6, 2.4, and 3.1 percent, respectively. If we are justified in assuming that we would have obtained similar values for the molasses samples reported here, 17 percent of the impurities are still undetermined.

Summary

The average of the total non-sucrose solids determined in the molasses samples examined constitutes 74 percent of the total impurities. Sodium, raffinose, acetate, galactinol, nitrate, and unknown nitrogen compounds are included in the remaining 26 percent. Up to 20 percent of the molasses impurities remain unidentified.

The impurity showing the greatest quantitative variation is chloride, which is of particular importance, since it is also one of the substances known to have an especially deleterious effect on sucrose crystallation.

Steffen molasses probably has a lower potassium and lactate content than straight house molasses although an insufficient number of samples have been examined to verify this.

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